DEVELOPING SAFE CULTURAL PRACTICES FOR WATER SPINACH (*IPOMOEA AQUATICA*) IN THE STATE OF GEORGIA, USA

by

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(Under the Direction of Timothy Coolong)

ABSTRACT

Water spinach (*Ipomoea aquatica*) is a tropical plant often grown and consumed in its native regions as a leafy green vegetable. In the U.S., it is classified as a noxious weed by the USDA due to its potential invasiveness in certain temperate regions. Georgia, USA has recently permitted imports and sales of water spinach into the state while cultivation is under consideration. The objective of this study was to develop safe cultural practices to provide for future Georgia growers due to a lack of current accessible growing information. An evaluation of nutrient requirements, planting and harvest periods, and fertilizer regimes was studied. Studies revealed that a 0.50 strength Hoagland's solution should be considered as a baseline fertility program in a hydroponic setting while high tunnel studies determined planting in May is an ideal time to plant water spinach outdoors to maximize yield.

INDEX WORDS: *Ipomoea aquatica*, water spinach, deep-water culture system, high tunnel, invasive species, growing practices, Hoagland's solution

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DEDICATION

To my parents, Donald and Christina, your love, guidance, and unwavering support has shaped me into the woman in am today. I am forever grateful for the strong foundation you have provided for me.

To my husband, Hunter, you have undeniably been my rock throughout this journey. Together, we can accomplish anything.

Most importantly, to my niece, Caroline – you can do anything you set your mind to. I will always be here to guide you, cheer you on, and hold your hand along the way.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Water spinach (*Ipomoea aquatica*) is an aquatic or semi-aquatic, tropical plant that belongs to the Convolvulaceae family. It naturally grows within tropical or subtropical areas in or near waterways where its roots can penetrate into moist soils and its spongy, hollow stems and leaves can trail into the water to float upon the surface (Austin, 2021). Water spinach is a plant native to China, but has historically been naturalized and cultivated in Africa, Asia, Australia, Pacific Islands and South America (Staples, 1996; Langeland & Burks 1998, p. 71).

There are two main varieties of water spinach that are recognized within Southeast Asia. The first, known as the red variety, has green to purple stems with dark green leaves that often contain a purple hue in the petioles and veins. This variety also produces purple to white flowers and can typically be found growing wildly in regions such as Malaysia and Thailand. The second is the white variety which has green to white stems and leaves with white flowers. The white variety can be divided into two sub-cultivars within the Philippines and Taiwan that are distinguishable of having either broad leaves or narrow, pointed leaves. (Westphal, 1993, p. 165)

Ideally, cultivation of water spinach should take place in areas where full sun is available, and temperatures are above 25 °C with an elevation over 500m. Otherwise, plant growth can be slow (Edie and Ho, 1969; Gangopadhyay et al., 2021). High levels of organic material, preferably within a pH range of 5.3-6.0 are best, but water spinach has adapted to a wide range of soil conditions (Westphal, 1993, p. 165). There are three main types of cultivation practices used when growing this crop. One approach is to grow in waterways such as rivers or ponds where seedlings or cuttings are grown atop the water's surface joined together in mats. Another method, known as wetland cultivation, takes place in saturated soil with a water level of 15-20cm. Seeds or cuttings are planted and cut 5-10cm above ground level when harvested. The final method, upland cultivation, takes place in soils that have not been heavily saturated with water. Rather, water spinach is cultivated in dry soil using the standard practices of a typical land plant. Seedlings, cuttings, or soaked seeds can be used with this method which can be broadcasted or sown into rows. It is important to note that this method can require supplemental fertilization of nutrients such as nitrogen and heavy supplemental irrigation. (Gangopadhyay et al., 2021; Westphal, 1993, p. 165)

This crop is commercially cultivated and widely consumed within regions of Southeastern Asia, Southern China, and India (Gothberg et al., 2002). It is considered a leafy green vegetable that is commonly harvested at a young stage where the tender tips and leaves of the plant can be boiled or steamed as well as incorporated into stir-fries, curries, stews, and soups. Along with the culinary aspects, this crop can also be used medicinally by utilizing the fresh plant or creating extracts, juices, and powders to address conditions such as high blood pressure and jaundice (Prasad et al., 2008). While human consumption is the primary motivation for growing water spinach, it can also be incorporated into supplemental animal feed to create a protein rich fodder within tropical countries such as Cambodia (Sambo et al., 2023). This animal fodder typically utilizes the older portions of the plant which are undesirable to the human market.

Outside of its native regions, water spinach has been known to escape cultivation and become an invasive species. Their hollow stems are sponge-like and can grow several feet long with many air passages present within them. This allows them to easily float throughout still or moving waterways. Once detached, prolific rooting is present at nodes can anchor the plant into soil or create a dense, floating mat within unwanted areas. A single plant can produce 175-245 labyrinth-type seeds which have the ability to float for an extended period of time and ultimately increasing the risk of unwanted dispersal (Edie and Ho, 1969). Sedentary plants can then vigorously grow and cover a large area in just a short amount of time. With the correct climate, 190,000kg of water spinach fresh weight biomass can be produced per hectare (84 tons per hectare) over a period of nine months (Langeland & Burks 1998, p. 70). Within the Philippines, water spinach was once considered the second greatest problem plant and can be known as a common to serious weed in many tropical areas (Holm et al. 1979). In the United States, water spinach has been repeatedly introduced into Florida since 1979 although it was recognized by the state as a threat even before introduction. It now has been naturalized in Florida waterways such as canals and ditches as well as the shallows of natural lakes with many attempts at eradication and not all being successful (Langeland & Burks 1998, pp. 70-71).

Within the U.S., water spinach is typically not readily accessible due to its classification as a federal noxious weed by the United States Department of Agriculture (USDA). Water spinach can legally be cultivated in California, Florida, Hawaii, and Texas, where the warm climates allow for the support and growth of this plant. Specific farms that grow water spinach are not widely published. Still, growers operate under strict guidelines and require permits to control where and how the crop is to be cultivated such as in greenhouses using only propagations and with careful management. Designated farms must adhere to these guidelines to prevent water spinach from moving into natural waterways where it can become invasive. The sale of water spinach can occur in states where cultivation is permitted and as of 2022, the

Georgia Department of Agriculture (GDA) has allowed for the sale of water spinach to take place within its state (Georgia Department of Agriculture, 2022).

The advocation for the sale of water spinach in the state of Georgia has taken place for many years with an Atlanta Asian supermarket collecting online signatures for the legalization of sales over a decade ago. Over the years, the Asian communities throughout the state have grown in population and the demand has become increasingly sought-after. Now that sales are permitted within Georgia markets, only the tops of the plants are sold, and a majority of their hollow stems are removed to mitigate chances of accidental propagation. (Lee, 2023). It is said that regulations are being put into place for the permitted cultivation of water spinach within Georgia, but little has been revealed as to when it will occur (Georgia Department of Agriculture, 2022).

As Georgia joins other states permitted to cultivate water spinach, it is important to be educated on the rapid growth and potential invasiveness that can occur. Aside from scientific literature, the current information available to growers is limited and a lack of guidance can pose a serious threat to the local ecosystems of Georgia. Along with understanding the potential threats, understanding the specific growing requirements is crucial for successfully cultivating water spinach and protecting the environment. Knowing the appropriate months to plant as well as soil and nutrient conditions will ensure this crop can thrive in designated locations. Without this knowledge, permitted growers may unintentionally contribute to the current threat that water spinach poses. Therefore, creating a detailed growing guideline can be essential to balancing the permitted cultivation with the protection of the environment.

Literature Review

Description

Water spinach (*Ipomoea aquatica*) is an herbaceous aquatic or semi-aquatic freshwater plant which belongs to the morning glory family (Convolvulaceae) (Rubatzky and Yamaguchi, 1997). *I. aquatica* is one of two aquatic species within its genus. Although hundreds of species are within the genus *Ipomea*, water spinach and sweet potato (*Ipomoea batatas*) are the only two species commonly grown as food crops (Wu et al. 2018). In addition to "water spinach", many common names are used when referring to *Ipomoea aquatica* such as kang kong, water convolvulus, and swamp morning glory (Austin, 2007). Water spinach is a diploid species (2n = 2x =30), though until recently there have been limited molecular analyses of the water spinach genome (Hao et al. 2021).

Morphologically, it is known for its long, hollow stems, which can trail or float along the surface of waterways. The ability to float can aid in dispersal in waterways as well as give the plant the ability to reach sunlight, out-competing other aquatic species that may be anchored in the soil. The main roots of the plant grow within moist soils along banks of waterways such as rivers or ponds. Stems are capable of growing adventitious roots present at nodes. It can be both an annual or perennial plant depending on the growing location and climate (Prasad et al., 2008). In tropical regions, where freezing temperatures do not occur, it may exist as a perennial. Stem colors range between purple to light green hues depending on the variety. Leaves are long petioled, can be lanceolate or hastate in shape, and alternate along the stem (Figure 1.1A, B). There are two main wild biotypes of water spinach that are recognized. The red type has green or purple stems, dark green leaves, and white or light purple flowers. The white type is characterized by its green or white stems, green leaves, and white flowers (Westphal, 1993 FNWD) (Figure 1.2A-C).

Habitat

I. aquatica grows best in warm, wet climates where temperatures reach above 25°C. Full sun and an elevation over 500m is also preferred (Edie and Ho, 1969; Gangopadhyay et al., 2021). *I. aquatica* grows best in areas with a soil pH range of 5.3-6.0 and high levels of organic material, especially when grown in non-aquatic environments (Westphal, 1993, p. 165). The wide adaptability of water spinach and rapid growth rate has also led to its classification as a weed species (Austin, 2007)

Reproduction

Reproduction can occur both sexually and asexually. Seed formation occurs during warm months when plants begin to flower, and a single plant can produce up to 245 seeds (Edie and Ho, 1969). Seeds have been known to have poor germination, especially when submerged underwater. Limiting seed production is an important part of *I. aquatica* management in the cultivated environment. *I. aquatica* flowers are cone-shaped and can be purple or white in color similar to others in the *Convolvulaceae* family. Although less is known regarding breeding of water spinach, empirical observations suggest that they are likely an outcrossing species with self-incompatibility similar to sweet potato (Martin, 1965). Because it is an outcrossing species, seed production in a greenhouse environment, without pollinators, is limited. Field cultivation may lead to seed production and subsequent invasiveness in environments where it may be naturalized as a weed (Austin, 2007). Asexual reproduction can take place when fragments of stems are removed and carried by water or animals. The hollow, spongey stems, have the ability to float through waterways, allowing vegetative fragmentation and ultimately is the primary means of reproduction in these environments. Roots growing at each node allow for many new independent plants to result from one single parent plant. (Patnaik, 1976; Edie and Ho, 1969).

Origin and Foreign Distribution

The native regions for *I. aquatica* are Asia, Africa, and the southwestern Pacific Islands. The true origin of water spinach is unknown but is thought to have originated in China or perhaps India (Austin, 2007). The first written record of *I. aquatica* dates back to at least 300 A.D. during the Chin Dynasty where water spinach seeds were germinated and grown on floating rafts (Edie and Ho, 1969). Distribution has occurred throughout tropical areas such as Southeast Asia, Australia, Africa, India, Sri Lanka, Fiji, the West Indies, South America, and Central America (Kirtikara and Basu 1952; Edie and Ho, 1969; Palada and Crossman, 1999). It has been suggested that *I. aquatica* was first cultivated in Southeastern Asia and since then has been widely grown commercially throughout China, Thailand, Indonesia, Philippines, Myanmar, and Bangladesh (Naskar, 1990).

Food and Medicinal Value

Widely consumed throughout tropical Asia, *I. aquatica* is usually incorporated into meals as a cooked vegetable. It is used within Asian cuisine due to leafy green vegetables being a necessity in most recipes. Typical greens such as cabbage, kale, or lettuce cannot grow during the hot summer in Southeast Asia, leaving water spinach as an important commodity as it can be grown and distributed locally during these months (Edie & Ho, 1969). Common culinary practices include frying, boiling, or steaming the tips of the plant, or incorporating them into soups, stews, stir fries, and curries (Westphal, 1993; Austin, 2007). Older, undesirable portions of water spinach can also be utilized as supplemental fodder for animals such as fish, ruminants, or chickens (Ali & Kaviraj, 2018; Kusumah & Pertiwi, 2021; Maung et al., 2020; Sambo et al, 2023)

There are also many medicinal uses associated with water spinach. It is considered a tonic due to having vitamins A, B, C, and E (Austin, 2007). Depending on the intended treatment, the fresh plant can be utilized, or it can be made into a juice, extract, powder, or poultice. It serves many purposes such as being a natural laxative, relieving headaches, or alleviating sleeplessness due to its calming effects (Burkill, 1936). The buds of the plant have been used to treat ringworm lesions and boils, poultices have been used to treat fevers and itch, and the juice of the plant can be as an emetic in instances such as arsenic poisoning (Burkill, 1936; Kapoor & Kapoor, 1980; Khare, 2007). Treatments for issues such as jaundice, high blood pressure, diabetes, and liver-related disorders can also be addressed with water spinach (Samuelsson et al., 1992).

U.S. Distribution

Within the U.S., *I. aquatica* has been naturalized in Florida, Guam, Hawaii and Puerto Rico (IRC, 2016; Stone, 1970; USDA, 2020). *Ipomoea aquatica* has been repeatedly reintroduced into Florida, since 1979 where multiple attempts of eradication have occurred, but not all have been successful (Langeland & Burks 1998). In 1998, three phenotypes were officially recorded existing in the wild in Florida. These types have similar descriptions to those found in Southeast Asia, with two types (red and white) being found floating in waterways and one type (white) found growing upland (Van and Maderia, 1998). *I. aquatica* is currently allowed to be cultivated in the states of Texas, Hawaii, Florida, and California. Since 2022, Georgia has approved permits for imports and sales of water spinach within the state (GDA, 2022). The availability of water spinach in Asian markets throughout the state has been advocated for over a decade by Asian community leaders of Georgia (Pearson, 2022). As of now, only the tops of the plants can be sold to reduce the risk of propagation and legal cultivation of water spinach in Georgia is still under review (GDA, 2022; Lee, 2022).

Cultivation

Typically, *I. aquatica* is grown using either dryland or wetland cultivation. With dryland cultivation, plants can be established by sowing seeds directly into the soil, through rooted cuttings or transplants. This method of cultivation is typical to most specialty crops where they are grown in soil with supplemental irrigation. Plants are given heavy applications of fertilizer once they have passed the seedling stage. In many regions, watering is done through furrow irrigation, with ditches on the sides of planted beds that are flooded regularly. However, overhead or drip irrigation has been shown to be effective as well. Given its status as an aquatic plant, it is no surprise that water spinach utilizes significant amounts of water. Empirical observations by the authors suggest that daily irrigation events using drip irrigation may be required to sustain growth. Given the extensive use of fertilizer and wet conditions there is often significant weed pressure in water spinach plantings. Further, given a lack of labeled herbicides (none in the US) significant hand weeding or cultivation will be required.

Wetland cultivation is more commonly practiced and is typically how the majority of the crop is produced in the summer months, particularly in areas where it has been traditionally cultivated. This method consists of flat fields with raised banks surrounding them. Fertilizer applications are similar to that of the dryland method and water is allowed to move throughout the field with a 12-hour period of no water between applications. Seedlings grow poorly when submerged underwater, so seeds are typically sown in dry soils and 30cm long stem cuttings are taken of the stems. *I. aquatica* seeds typically have a germination rate of less than 60% and germination rate can vary between seed types of different colors or seedcoats, with black seed types having the most success. Development will begin 2-3 weeks from the sowing date and harvest can occur as few as 20 days later (Westphal, 1993). For this reason, utilizing cuttings is an advantageous route for cultivation, if labor is available. This production method is somewhat similar to how sweet potatoes are commonly grown in the U.S., using vegetative propagation and cuttings (taken above ground) or slips (pulled from below ground) (Rubtazky and Yamaguchi, 1997). Cuttings are routinely planted in the field where they can be flooded with 3-5cm of water and initiate rooting into the soil. Water depth can then slowly be increased to 15-20 cm as plants establish themselves in the field.

Using flooding can reduce weeding, much like lowland rice production, (Coolong, 2013; Edie and Ho, 1969). However, extensive weeding may be required when producing the cuttings for the field. Another less common method of cultivation is to utilize waterways such as rivers or ponds where seedlings or cuttings are placed into mats and grown atop the surface of the water. With this method, irrigation or flooding is not necessary, but fertilizer applications cannot be controlled (Gangopadhyay et al., 2021). During the late summer months, flowers will begin to form on plants, growth will tend to slow, and harvesting will end (Edie and Ho, 1969).

Deep Water Culture Systems

Due to the aquatic nature of water spinach, deep water culture (DWC) systems have been identified as a way to produce the crop in a greenhouse setting. Further, production in a greenhouse would limit the ability to become naturalized in the environment as outcrossing ability is reduced and vegetative parts of the plant are in a confined space. The DWC system, also known as floating or raft systems, was developed by Dr. Merle Jensen in the 1980s (Jensen and Collins, 1985). Since then, it has been a common hydroponic system adopted by both commercial and small-scale growers. This production system allows plant roots to be submerged underwater while the shoot portion of the plant is supported and divided from the water by a raft or covering. The underwater root portion of the plants are typically provided nutrient solutions and oxygen to ensure proper nutrition and growth. DWC systems hold water in tanks or reservoirs that can be many shapes or sizes depending on the production scale and depths vary between 2 to 12 inches. The raft or covering that the shoot portion of the plant is grown on can be created out of a variety of materials. Typically, commercial rafts are made out of polystyrene resins which can float on the surface of the water, ensuring that roots are always consistently submerged underwater. Coverings that do not float on the surface of the water can also be used and are typically made from a plastic material. The nutrient solution provided to the plants is added into the water and circulated using some type of pump which also provides roots with dissolved oxygen (Mullins et al., 2023; Velazquez-Gonzalez et al., 2022).

Invasive species

Ipomoea aquatica is classified as an invasive species in Bangladesh, India, Israel, Mexico, and the United States (Randall, 2017; USDA, 2020). Its ability to float along the surface of waterways combined with prolific rooting at nodes allows for water spinach to easily spread into unwanted areas quickly. Tropical areas with high humidity allow for the perfect environment to host water spinach and in these correct conditions, 84 tons of fresh weight per hectare of biomass can be produced over a nine-month period (Harwood and Sytsma, 2003; Langeland & Burks, 1998). Established plants then can create impenetrable floating mats consisting of stems intertwining with one another. This ultimately shades the native plants beneath the water's surface while also outcompeting native plants present on the surface and displacing wildlife. Once established, water spinach is difficult to eradicate. As mentioned previously, Florida has had multiple attempts at eradicating water spinach, and it is now currently naturalized within the state. Aquatic herbicides have been utilized to control water spinach, but the results were only temporary. Other broad-spectrum herbicides such as Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) have been shown to control water spinach but also consequentially affect native vegetation which is unacceptable from a conservation standpoint (Schardt & Schmitz, 1990). According to the USDA, 9% of the United States was estimated to be suitable for the establishing water spinach.

Pests

Non host-specific problems include *Pythium* root rot, *Cercospora* leaf spot, root-knot nematodes (*M. incognita* and *M. javanica*) and aphids (*Aphis* sp.). Two polyphagous lepidopterans, *Diascrisia strigulata* and *Spodoptera litura* have been reported to feed on water spinach. The pathogen *Albugo candida* also can be a pest of water spinach. (Westphal, 1993; Dueñas-López, 2023; Austin, 2007).

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FIGURE 1.1 (A, B): Two selections of *Ipomoea aquatica* with varying leaf structure. A) Lanceolate leaf type more common in Southeast Asia. B) Hastate leaf type that is more common in China.

FIGURE 1.2 (A, B, C): Floral phenotypes of *Ipomoea aquatica*. Flower colors range from A) white with deep purple centers, B) all white, and C) white with pink centers.

CHAPTER 2

MACRONUTRIENT UPTAKE AND ACCUMULATION FOR WATER SPINACH (*IPOMOEA AQUATICA*) GROWN IN A DEEP-WATER HYDROPONIC CULTURE SYSTEM[1]

[1] Bohensky, S. and Coolong, T. (2025) To be submitted for publication in HortTechnology.

Abstract

Water spinach (*Ipomoea aquatica*) is widely cultivated throughout parts of Southeast Asia as a leafy green. Despite being considered a noxious weed by the United States Department of Agriculture, it has been recently allowed to be purchased and sold in the state of Georgia, USA. While cultivation is still prohibited, there are plans that may allow for production in controlled environments such as greenhouses or high tunnels. However, information on production practices is limited. To evaluate the response of water spinach to different fertilizer regimes, two selections of water spinach displaying distinct phenotypes were greenhouse grown in a 0.50 and 0.25 strength Hoagland's nutrient solution using a deep-water culture system. Plants were grown for 61 days after transplanting (DAT) with entire plant samples taken at 21, 31, 41, 51, and 61 DAT to determine root and shoot biomass, nutrient concentrations, and total nutrient accumulation. Potassium was the most abundant nutrient, reaching concentrations of nearly 8.3% in the aboveground portion of plants at 21 DAT. Nitrogen was the next most abundant nutrient measured. Nutrient accumulation for all macronutrients was linear in both shoots and roots throughout growth. While aboveground biomass increased when grown in the 0.50 strength Hoagland's solution compared to the 0.25 strength solution, selection 2, a lanceolate leaf type, responded more positively than selection 1, a hastate leaf type, to the increased nutrient concentrations in the 0.50 strength fertilizer solution. These results suggest that water spinach growth and nutrient uptake were significantly improved using the 0.50 strength Hoagland's solution in a deep-water culture system.

Introduction

Water spinach (*Ipomoea aquatica*) is an aquatic or semi-aquatic plant that is widely cultivated and consumed throughout India, Southeast Asia, and Southern China (Edie and Ho 1969; Rana and Brar 2018). It is consumed as a leafy green vegetable with tender shoots being harvested for consumption. Water spinach flourishes in tropical and subtropical areas, where air temperatures exceed 25 ^{o}C, rooting along the outer edges of waterways with long, hollow stems that float along the water's surface (Austin 2007; Edie and Ho 1969; Gangopadhyay et al. 2021). The young, tender tips and leaves of the plant can be utilized in various dishes such as stir-fries, stews, and curries, but can also be boiled or steamed to be eaten on their own. Water spinach occupies an important niche due to its ability to grow during the hot summer, when many leafy greens such as lettuce (*Lactuca sativa*) or *Brassica* species cannot survive (Edie & Ho 1969). Juices, poultices, extracts, and powders may also be created from the stems and leaves of water spinach to be used medicinally for treatments or ailments such as headache relief, ringworm, or high blood pressure (Burkill 1936; Khare 2007; Samuelsson et al. 1992). As the plants grow larger and stems are less desirable for human consumption, the biomass of *I. aquatica* can then be turned into supplemental fodder for livestock such as ruminants and chickens (Ali and Kaviraj 2018; Kusumah and Pertiwi 2021; Maung et al. 2020; Sambo et al. 2023).

Water spinach is also commonly referred to as swamp morning glory, kang kong, and water convolvulus and is cultivated both on land and in aquatic environments (Austin 2007; Snyder et al. 1981). Plants cultivated on land are typically irrigated using furrow/flood irrigation. In wetland systems flooded fields are maintained with a water depth of approximately

15-20cm (Rubtazky and Yamaguchi 1997; Snyder et al. 1981; Westphal 1993). A less common, but still utilized method, is to cultivate seedlings or cuttings by placing them in woven mats and floating them on the surface of waterways (Gangopadhyay et al. 2021). While fertilizer application cannot be controlled in free waterways, water spinach has been reported to effectively grow under low nutrient levels such as in eutrophic waters (Maruo et al. 2004).

In regions such as the United States, Mexico, and Bangladesh, *I. aquatica* is classified as an invasive species (Chilton 2017; Randall 2017; USDA 2020). The hollow stems of the plant float on surfaces of waterways, while their nodes prolifically root. When fragments of stems are broken from the main plant, they can float downstream and anchor themselves in unwanted areas. Established plants are challenging to eradicate and can produce large amounts of biomass over short periods of time (Harwood and Sytsma 2003; Langeland and Burks 1998).

While the U.S. has classified *I. aquatica* as a federal noxious weed, it can be legally grown in Florida, California, Texas, and Hawaii under strict guidelines intended to limit plant spread from cultivated areas (Chilton 2017; USDA 2020). Recently the state of Georgia, USA allowed for the legal import and sale of water spinach, though production in prohibited at the time of this publication (Lee 2023; GDA 2022). However, there are plans to allow regulated cultivation within the state, encouraging the use of protected agriculture (personal communication GDA).

Maruo et al. 2004 reported that macronutrient absorption rates for water spinach were relatively unaffected by nutrient concentrations in a hydroponic system, suggesting that water spinach may not benefit from elevated fertilizer levels. However, other reports suggest that water spinach foliage may contain from between 3.5 to 4.5% nitrogen (N) on a dry-weight basis and can have the ability to remove 446 $kg \cdot ha^{-1}$ N annually from areas in production in Florida, USA (Snyder et al. 1981). While this large amount of N removal is a function of large biomass production and N concentration, water spinach may respond positively to additional fertilization. Furthermore, high levels of potassium (K) (>5% dry weight) have been reported in the foliage of water spinach (Umar et al. 2007). This suggests that the plant can accumulate significant quantities of macronutrients when available. Due to the lack of clearly defined fertilizer recommendations for water spinach in the US, the purpose of this study was to determine the response of two selections of water spinach to two fertilizer regimes in a deep-water culture system, similar to what may be used by producers in Georgia, USA.

Materials and Methods

Experimental Setting

Two independent experiments were conducted in a greenhouse in Watkinsville, GA, USA (lat. 33° 5'N, long. 83° 3'W) from 23 May to 23 July 2023 and 2 Aug. to 2 Oct. 2023 using a deep-water culture system. Two unnamed selections of water spinach were utilized which are hereafter noted as selection 1 (Gaea's Blessing, Austin, TX, USA) and selection 2 (Chiatai seed, Bangkok, THA). Selection 1 had a more hastate leaf type and is more commonly utilized in China (Figure 2.1A), while selection 2 has a lanceolate leaf type and is more commonly found throughout southeast Asia (Figure 2.1B) (Westphal 1993).

The temperature and relative humidity (RH) of the greenhouse were monitored at canopy height hourly (VP4; Meter Group Inc., Pullman WA, USA). Average environmental conditions during both studies were similar. In the first study, average air temperatures during the day and night were 27.2 °C and 20.8 °C, respectively. Average RH during the day and night were 64% and 81%, respectively. Light levels measured at noon averaged 958 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and ranged from a minimum of 197 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to maximum of 1398 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In study two, average air temperatures during the day and night were 28.5 ^{o}C and 20.7 ^{o}C, respectively. Average RH during the day and night were 65% and 83%, respectively. Light levels measured at noon averaged 940 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and ranged from a minimum of 113 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to maximum of 1296 $\mu mol \cdot m^{-2} \cdot s^{-1}$ depending on cloud cover at the time the measurement was taken.

Seeds were sown into pre-soaked 2.54 cm diameter rockwool cubes (Grodan, Roermond, NLD) then covered with vermiculite for germination. Rockwool cubes were placed into plastic trays and inside the greenhouse with daily irrigation. Greenhouse temperature set points were 28/20 ^{o}C (day/night). Seedlings were grown for 28 d then placed into plastic net pots (4.7 cm W x 5.1 cm D) and transferred to 37.9 L plastic containers (Rubbermaid Inc. Wooster, OH, USA) filled with 28 L of well water. Seedlings were not fertilized prior to placement in the nutrient solutions to ensure that preplant fertilizers did not influence the nutrient solution treatments. The well water was analyzed for nutrient content at the start of the experiment (Table 2.1). Fifteen seedlings were placed equidistantly within holes drilled in each lid. An aquarium air stone attached to an air pump (Active Aqua; Hydrofarm, Petaluma, CA, USA) was placed inside each plastic container to provide aeration to the plants and nutrient solution. Throughout the experiment, supplemental water was added to containers as needed to maintain the 28 L volume. Solution pH was maintained at approximately 6.7-6.9 at the start of growth using pH down (pH down when necessary; General Hydroponics, Santa Rosa, CA, USA). At planting, a 0.25 or 0.50-strength Hoagland's no. 1 solution (Hoagland and Arnon, 1950) was added to each tub (Table 2.1). The experiment was arranged as a factorial randomized complete block design with two varieties and two nutrient solutions replicated four times. Each replicate (tub) contained 15 plants at the start of the experiment. At planting, 20 seedlings from each selection were dried in a forced air oven set at 60 °C until a constant weight was achieved to determine root and shoot biomass and nutrient concentrations at planting.

Plants were grown for 21 d in their corresponding nutrient solution at which time two plants were removed from each container to determine biomass and nutrient content. Each container was thoroughly rinsed with well water and nutrient solutions were completely replaced, and the remaining plants placed into tubs. Plants were removed, and nutrient solutions were replaced every 10 d until 61 d after transplant (DAT). At the final harvest, seven plants remained, and all were harvested for dry biomass and nutrient concentration determination. Nutrient solutions were sampled at the beginning and end of each cycle using 20 mL scintillation vials (HDPE; Thermo-Fisher Scientific, Waltham, MS, USA), and stored at 4 °C until analysis (Table 2.1).

Nutrient Analysis

At harvest, the shoots and roots of each plant were separated and roots removed from the rockwool fibers and thoroughly washed with well water after sampling to ensure no excess nutrient solution was included in the analyses. Fresh tissue samples were placed in a forced air oven set at 60 ºC until a constant weight. Drying times varied based on sample size at each growth stage. Dried plants (roots and shoots) were weighed and coarsely ground. Two plants per replicate were sampled during growth and seven at the final harvest (61 DAT) to obtain average plant dry weight and nutrient concentrations. Dried plant material was analyzed by the University of Georgia Agriculture and Environmental Services Laboratory (Athens, GA, USA). In brief, coarsely ground plant material was ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) and passed through a 20-mesh screen. Samples were digested using EPA Method 3052 (USEPA, 1995).

Samples of the hydroponic solutions were filtered using a 0.45 µM PTFE membrane (Thermo Scientific™ Choice™ Polypropylene Syringe Filters) and acidified using 2% (V/V) high purity nitric acid (HNO3) (Certified ACS Plus, Fisher Chemical) before analysis. Hydroponic solutions and plant tissues were analyzed for multiple elements following EPA Method 200.8 (Creed et al., 1994) by Inductively Coupled Plasma - Optical Emission Spectroscopy (Spectro Arcos FHS16; Spectro Amertek USA, Wilmington, MA, USA).

Statistical Analysis

Macronutrient concentrations of dried shoot and root tissue were multiplied by the average plant dry weight at each sampling time to determine nutrient accumulation. Statistical analysis was conducted using R and SAS (Ver. 9.4, SAS Institute, Cary, NC, USA) statistical computing software. Nutrient concentration, plant biomass, and nutrient accumulation were analyzed using the linear mixed model and regression procedures. When statistical differences existed in the GLM procedure ,Tukey's Honest Significant Difference (Tukey HSD) test ($P < 0.05$) was performed when necessary.

Results and Discussion

Biomass

There was a linear increase in biomass throughout the entire study for the two water spinach selections and both nutrient solutions. For both selections, the 0.50 strength Hoagland's solution treatments resulted in greater rates of biomass accumulation per plant compared to the 0.25 strength (Figure 2.1A, B). The two selections responded differently to fertilizer solution strength, however. The slopes of the trend lines for selection 1 were Shoot DW = 0.550*DAT and Shoot DW = 0.467*DAT for the 0.50 and 0.25 strength nutrient solutions, respectively. However, the slope of the trend lines for selection 2 were Shoot DW = 0.745*DAT and Shoot

DW = 0.374*DAT for the 0.50 and 0.25 strength nutrient solutions, respectively. This suggests that selection 2 responded more positively to increased nutrient solution fertility levels than selection 1. A similar trend was observed for root biomass production as well, with the slope for the trendline more than doubling between the 0.25 and 0.50 strength nutrient solutions for root biomass in selection 2. A smaller increase was observed for root biomass between the different nutrient solutions for selection 1. This indicates that there are differences in growth responses to fertilizer concentrations among the different types of water spinach grown. While water spinach may be successfully grown at fertility levels that are lower than those used in the current study, our results suggest that selection 2, which had a lanceolate leaf, responded favorably to increased fertilization (Liu et al. 2022). The average shoot (leaf and stem) dry biomass of selection 1 and 2 were 33.6 g/plant and 45.4 g/plant, respectively at 61 DAT, which are similar to those obtained in outdoor trials in Florida over an 81-d period when yields are estimated on a per plant basis as well as in greenhouse cultivation using a nutrient film hydroponic system (Pinker et al. 2004; Snyder et al. 1981). Shoot biomass ranged from 6.8 to 7.9 times that of root biomass depending on the selection and nutrient solution (Figure 2.1A, B). Liu et al. (2022) reported an approximate 1:1 ratio of root and aboveground biomass on a dry weight basis. However, that study was conducted with lower nutrient concentrations which may have resulted in a greater root production than shoots. Biomass continued to increase throughout the duration of the study, with no indication that growth would slow or plateau during the 61 d that it was conducted. Because water spinach can grow as a perennial and be harvested all year long in a tropical climate, continued growth in the greenhouse environment may be expected (Rana and Brar 2017).

Nutrient Concentrations

Nitrogen concentrations in shoots and roots were affected by both sampling time and nutrient solution concentration (Table 2.2). Regardless of the treatment, N levels in shoots and roots increased after being placed in the nutrient solution (0 to 21 DAT), which was followed by a steady numerical decrease in N concentration. For selection 2, there were significant decreases in shoot and root N concentrations in both nutrient solutions at 21 and 61 DAT, unlike selection 1, which only had a significant reduction in shoot N concentrations at 61 DAT when grown in the 0.25 strength Hoagland's solution. This suggests that nutrient solution strength may have impacted N concentration in selection 2 more so than selection 1, which is supported by biomass results. While studies have been conducted comparing ammonium to nitrate sources of N (Xiang et al., 2020), there is limited information regarding N concentrations in shoot or root tissue of water spinach. Nonetheless, shoot levels of N were within the normal expected range for early and mid-season foliage, before root enlargement, for sweetpotato (*Ipomea batatas*) (Bryson et al. 2014).

Potassium is often the most abundant mineral element present in plants on a weight/weight basis. It is required for many vital plant functions and is often the most abundant macronutrient found within the foliage of water spinach (Amir et al., 2021; Umer et al.,2007). In the present study, K was also the most abundant nutrient measured (Table 2.3). Shoot K concentrations at 21 DAT were 7.03% and 8.29% in shoot tissue for selections 1 and 2, respectively. Shoot K concentrations later decreased to between 3.10% and 4.86% from 41 to 61 DAT. Umar et al. (2007) reported K concentrations of 5.4% from water spinach growing aquatically in Nigeria, while other studies have reported K concentrations of less than 1% (Amir et al., 2021). Shoot or root K concentrations were not affected by nutrient solution strength when comparing a single sampling date for either selection of water spinach. Although not compared statistically, K concentrations were notably greater in shoot tissue compared to roots beginning at the 51 DAT sampling time, suggesting that K was being actively translocated to the foliage to support growth (Bryson et al. 2014) for selection 2 (Table 2.2).

Calcium concentrations in roots and shoots were affected by interactions between nutrient solution strength and sampling time (Table 2.4). No differences existed between nutrient solution strength and Ca concentrations in shoots at a given sampling time. The same relationship occurred in roots, except for those sampled at 61 DAT from selection 1, where roots grown in the 0.25 strength Hoagland's solution were 0.62% Ca, while those from plants grown in the 0.50 strength solution had Ca concentrations of 0.92%. Calcium concentrations in the foliage tended to be greater than in the roots and were greater in the current study than previously reported (Amir et al. 2021; Umar et al. 2007). It should be noted that the samples utilized in those studies were not obtained from plants grown in a nutrient solution, which may explain the difference. Our results are comparable to those obtained for foliar analysis of sweet potato grown specifically for production of shoot tissue (Ishiguro et al., 2004).

Root and shoot concentrations of phosphorous (P), magnesium (Mg) and sulfur (S) were also determined. These nutrients were in lower abundance than N, K, and Ca and had fewer differences in concentrations between treatments or selections and are available as supplemental (Supplemental Table 2.1).

Total Nutrient Accumulation.

The total N content in plants (roots and shoots) increased linearly for both water spinach selections and nutrient solutions. The rate of N uptake had a greater slope for both selections grown in the 0.50 strength Hoagland's solution (Figure 2.3A, B). The rate of total N accumulation was greater in selection 2 compared to selection 1, which was likely a function of the increased biomass associated with selection 2. This indicates that selection 2 may have a higher capacity for N accumulation and in production systems may require more frequent or higher N applications for optimal productivity. Total N accumulation in selection 2 was predicted to be 1.9 g/plant (Figure 2.3B) at 61 DAT. Using a population of 64,555 plants/ha (26,136 plants/acre) total N removal would be approximately 122 $kg \cdot ha^{-1}$ for the 61-d duration of this study. This is comparable to N removal for a similar period of time for studies conducted in Florida, USA (Snyder et al., 1981). The study conducted in Florida had a lower N concentration in plants but used a higher plant population per hectare. Water spinach has been shown to be effective at removing nitrates from effluent water (Enduta et al., 2011) and our results suggest that it may remove large amounts of N from the environment over a relatively short period of time due to its ability to produce large amounts of biomass and accumulate N in plant tissue at concentrations of 2% to 3% dry weight over a period of two months. These results suggest that while both selections accumulated more N when grown in the 0.50 strength Hoagland's solution, selection 2 could accumulate greater total amounts of N over the 61-d growing period. Plants sampled at 41-61 DAT in selection 2 accumulated approximately 1.6x the amount of N compared to selection 1.

Total K accumulation in plants increased linearly over time for both water spinach selections (Figure 2.4A, B). Selection 2 had notably greater K accumulation than selection 1 plants. However, this may be due to greater biomass production in selection 2 (Figure 2.2A, B) rather than greater K concentrations at 61 DAT. The difference in the slope of K accumulation between plants grown in the 0.50 and 0.25 strength Hoagland's solution was notable (Figure 2.4A, B). Plants grown in the 0.50 strength Hoagland's solution had a slope that was nearly double that of those grown in the 0.25 strength solution. The slope for K accumulation in plants was also greater than that of N as well (Figure 2.3A, B). Prior studies have indicated that K is often present in the greatest amount of any of the plant macronutrients in water spinach (Amir et al. 2021; Rana and Brar 2017; Umer et al. 2007). While little information is available regarding K removal, based on the rate of K uptake per plant our results suggest that 155 $kg{\cdot}ha^{-1}$ of K could be removed in a hydroponic or aquatic production. The slope of K uptake was steeper for both selections grown in the 0.50 strength Hoagland's solution (Figure 2.3A, B). This suggests that the selection 2 plants (lanceolate leaf) may remove more K from the growing environment than selection 1 plants.

Total Ca uptake in plants increased linearly over time. As with N and K, the accumulation of Ca was greater for plants grown in the 0.50 strength solution than the 0.25 strength solution (Figure 2.5A, B). Interestingly, the difference in slope between the 0.50 and 0.25 strength solutions was less than the difference in slope between the two solutions when estimating biomass. This suggests diminishing returns of providing additional Ca above the 68 ppm that was present on average in the 0.25 strength solution (Table 2.1), while the same was not observed with additional N and K applications.

Nutrient accumulation trends were also determined for P, Mg, and S (Supplemental Figures 2.1-3A, B). Due to lower accumulation of these nutrients and minimal treatment differences they have been included as supplemental data. The slope for predicted uptake of P and Mg were similar for both selections and nutrient solutions. The slope for S accumulation in selection 1 (Supplemental Figure 2.3A) was roughly half that of the slope for selection 2 (Supplemental Figure 2.3B), though differences in S accumulation between nutrient solutions were minimal.

Conclusion

Due to restrictions on production in the U.S. and the methods of production in other countries, there are no widely recognized guidelines for hydroponic nutrient solutions for the production of water spinach in greenhouse environments. The results of this study suggest that there are differences between water spinach selections in response to nutrient solutions and that the lanceolate-leaf type responded more to increased nutrient concentrations in the 0.50 strength Hoagland's solution. Differences in nutrient uptake and biomass production between the two nutrient solutions suggest that producers should consider the 0.50 strength Hoagland's solution as a baseline for hydroponic production of water spinach and that it may likely benefit from increased concentrations of some nutrients. Our results also suggest that this crop has significant potential for nutrient removal from deep water culture systems.

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TABLE 2.1: Average starting concentrations in parts per million (ppm) of macronutrients nitrogen (N), phosphorous (P), potassium (K), calcium (Ca) magnesium (M*g*) and pH of the well water and 0.25 and 0.50 strength Hoagland's no. 1 solutions used in this study. Concentrations are the average of the six initial solutions for each water change.

	N	P	K	Ca	Mg	pH
			(ppm)			
Well Water	2.1	<0.02	1.9	7.3	1.5	6.7
0.50 Strength[z]	118.4	16.3	131.8	107.3	28.4	6.8
0.25 Strength	61.5	10.2	67.9	61.2	10.2	6.9

[z]Nutrient solution: $Ca(NO_3)_2 \cdot 4H_2O$, KNO_3, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, H_3BO_3, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $H_2MoO_4 \cdot H_2O$, and Sequestrene 330.

TABLE 2.2: Nitrogen concentration in percent dry weight (DW) of shoots and roots at different days after transplant (DAT) for hydroponically grown water spinach.

			DAT					
			0	21	31	41	51	61
		Nutrient Solution Strength	DW(%)					
Selection 1	Shoot	0.5	1.28 d[i]	4.33 ab	3.47 abc	2.96 abc	3.24 abc	2.94 bc
		0.25	1.28 d	4.53 a	3.63 abc	2.72 dc	2.71 dc	2.50 dc
	Root	0.5	1.28 b	4.25 a	3.14 a	2.75 ab	2.96 ab	3.36 a
		0.25	1.28 b	3.95 a	3.25 a	2.53 ab	2.54 ab	2.79 ab
Selection 2	Shoot	0.5	1.35 f	4.97 a	4.00 abc	3.42 cde	2.95 cde	2.47 g
		0.25	1.35 f	4.77 ab	3.89 abc	3.69 bcd	2.71 de	2.34 ef
	Root	0.5	1.35 e	4.83 a	3.68 a-d	2.69 cde	2.74 cde	2.28 de
		0.25	1.35 e	4.46 ab	4.07 abc	3.22 bcd	2.41 de	2.13 de

[i]Numbers followed by the same letter(s) indicate no significant difference according to the Tukey HSD test (P<0.05) when comparing shoot or root N concentrations among the different nutrient solutions and sampling times for a single selection.

TABLE 2.3: Potassium concentration in percent dry weight (DW) of shoots and roots at different days after transplant (DAT) for hydroponically grown water spinach.

			DAT					
			0	21	31	41	51	61
		Nutrient Solution Strength	DW(%)					
Selection 1	Shoot	0.5	1.75 e[i]	6.74 a	5.77 abc	4.64 a-d	4.48 a-d	4.86 a-d
		0.25	1.75 e	7.03 a	6.01 ab	3.10 de	3.38 b-e	3.30cde
	Root	0.5	1.75 ab	4.49 a	1.74 ab	1.50 ab	1.81 ab	2.54 ab
		0.25	1.75 ab	3.70 ab	1.41 b	1.09 b	1.09 b	1.34 b
Selection 2	Shoot	0.5	1.65 c	7.50 a	6.51 a	4.41 b	4.04 b	3.54 bc
		0.25	1.65 c	8.29 a	8.28 a	3.73 b	4.12 b	3.16 bc
	Root	0.5	1.65 cd	6.06 ab	3.00 bcd	2.54 cd	1.50 d	1.72 cd
		0.25	1.65 cd	6.68 a	4.84 abc	3.32 bcd	3.93 d	1.41 d

[i]Numbers followed by the same letter(s) indicate no significant difference according to the Tukey HSD test ($P<0.05$) when comparing shoot or root K concentrations among the different nutrient solutions and sampling times for a single selection.

TABLE 2.4: Calcium concentration in percent dry weight (DW) of shoots and roots at different days after transplant (DAT) for hydroponically grown water spinach.

			DAT					
			0	21	31	41	51	61
		Nutrient Solution Strength	DW(%)					
Selection 1	Shoot	0.5	1.37 bc[i]	1.78 ab	1.62 abc	1.49 abc	1.56 abc	1.63 abc
		0.25	1.92 a	1.92 a	1.69 abc	1.27 c	1.40 bc	1.43 abc
	Root	0.5	1.37 a	0.59 cd	0.81 bc	0.87 bc	0.77 bc	0.92 b
		0.25	1.37 a	0.46 d	0.58 cd	0.63 bcd	0.56 cd	0.62 cd
Selection 2	Shoot	0.5	1.04 b	1.76 a	1.83 a	1.53 b	1.56 ab	1.48 ab
		0.25	1.04 b	1.85 a	1.89 a	1.12 b	1.40 ab	1.40 ab
	Root	0.5	1.04 a	0.57 a	0.84 a	0.92 a	0.64 a	0.71 a
		0.25	1.04 a	0.52 a	1.11 a	0.91 a	1.17 a	0.61 a

[i]Numbers followed by the same letter(s) indicate no significant difference according to the Tukey HSD test ($P<0.05$) when comparing shoot or root Ca concentrations among the different nutrient solutions and sampling times for a single selection.

FIGURE 2.1 (A, B): Two selections of water spinach (*Ipomoea aquatica*) with varying leaf structure. A) hastate leaf type that is more common in China, selection 1. B) lanceolate leaf type that is more common in southeast Asia, selection 2.

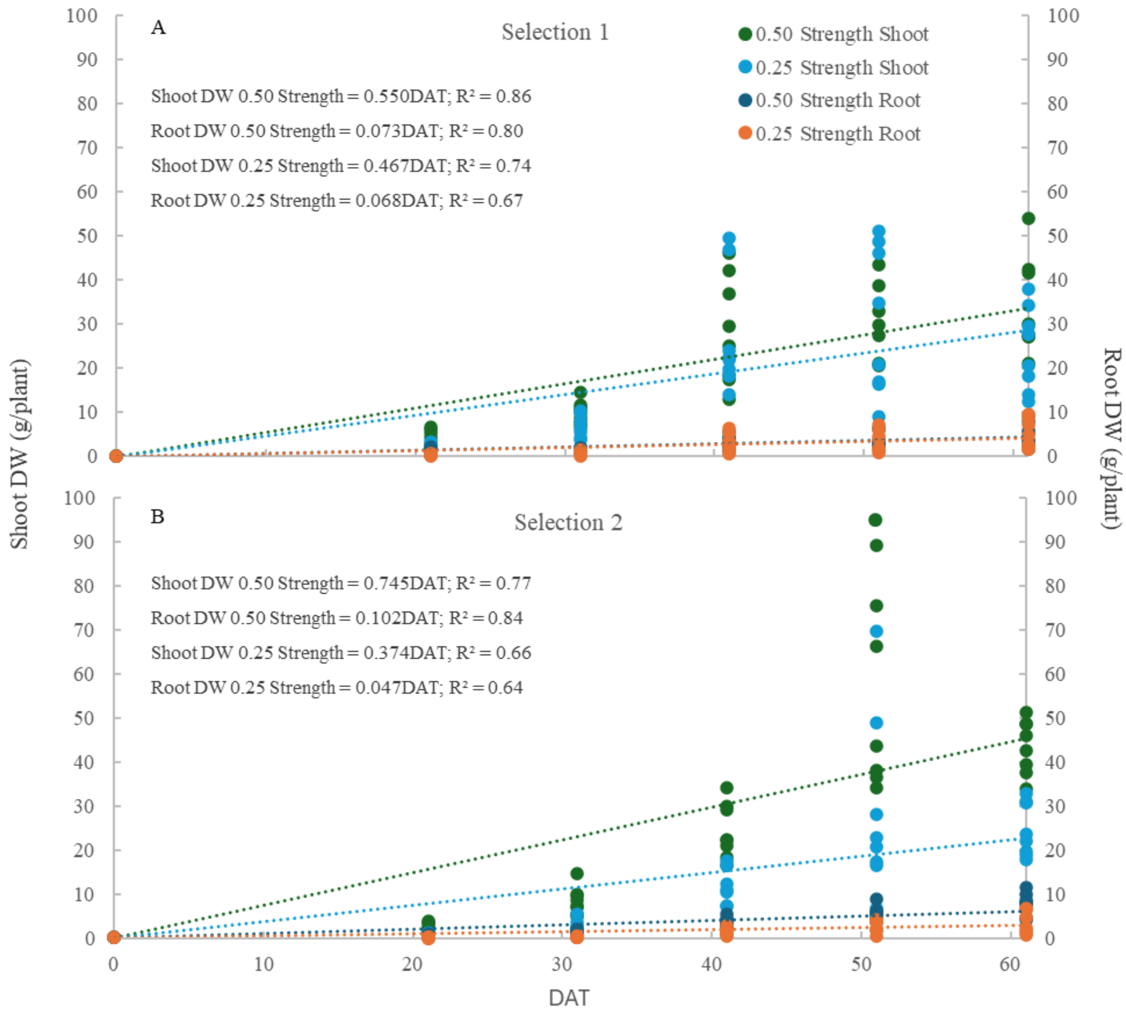


FIGURE 2.2 (A, B): Shoot and root biomass (g/plant) on a dry weight basis for water spinach (*Ipomoea aquatica*) selection 1 (A) and selection 2 (B) grown in 0.50 and 0.25 strength Hoagland no. 1 solutions.

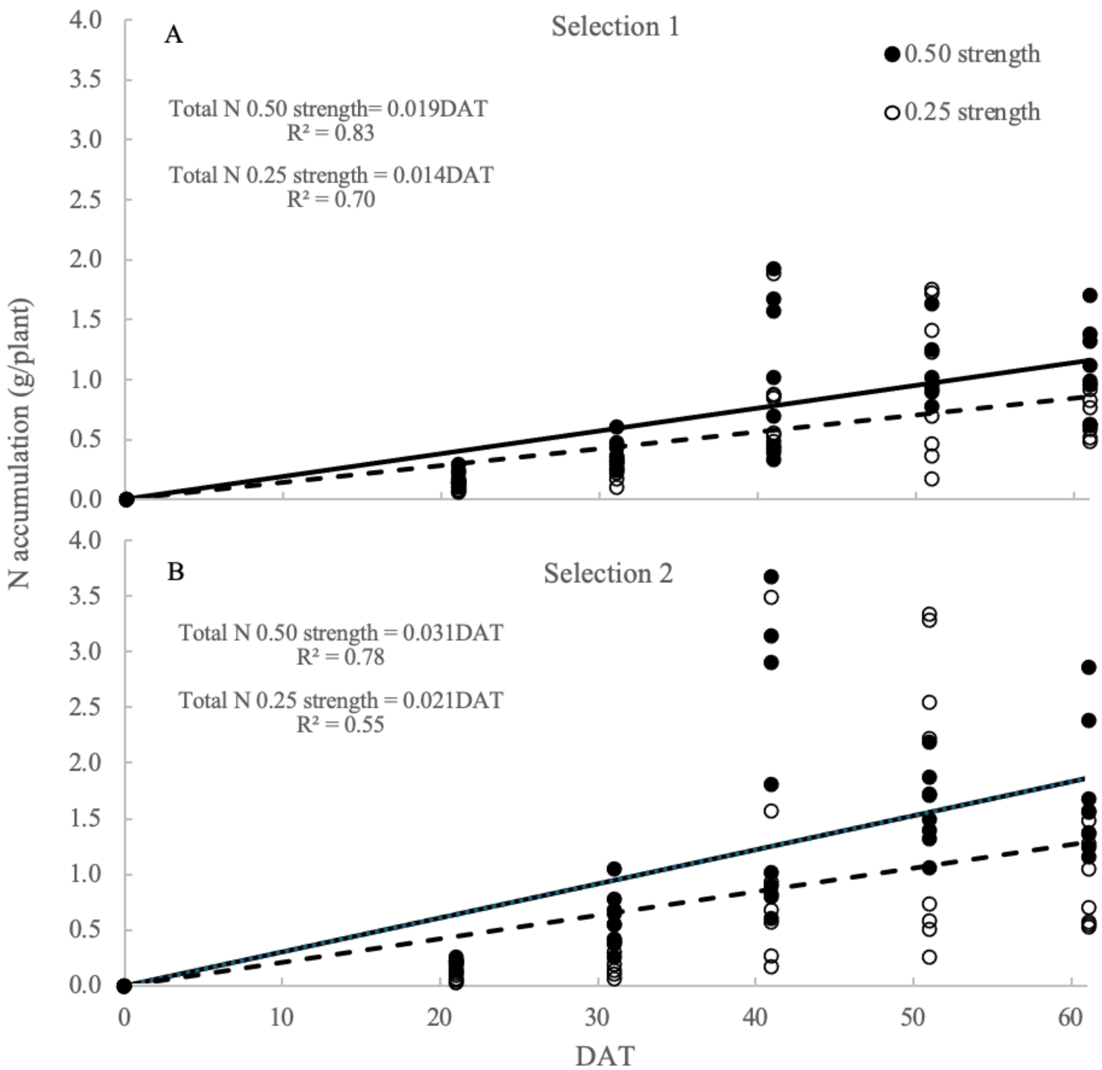


FIGURE 2.3 (A, B): Total nitrogen (N) uptake for root and shoot tissue on a dry weight basis for water spinach (*Ipomoea aquatica*) selection 1 (A) and selection 2 (B) grown in 0.50 and 0.25 strength Hoagland no. 1 solutions.

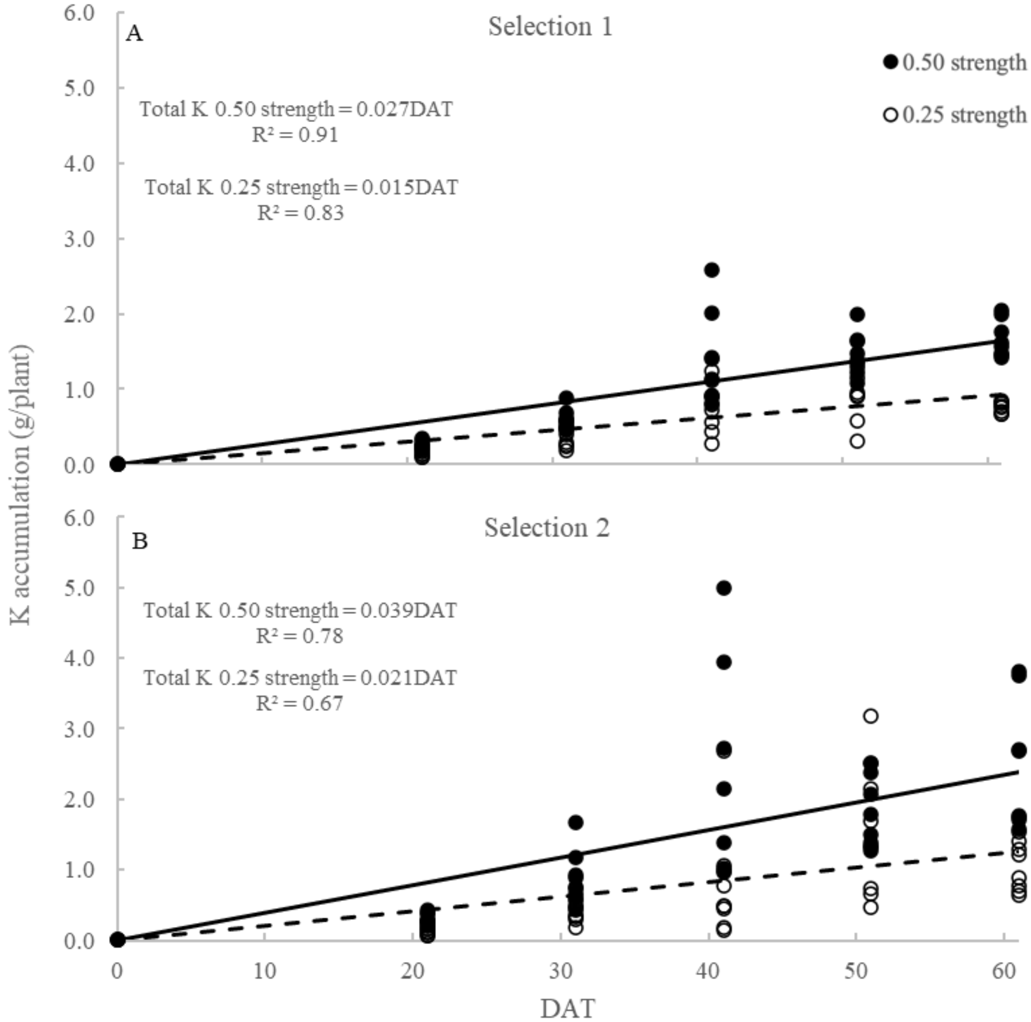


FIGURE 2.4 (A, B): Total potassium (K) uptake for root and shoot tissue on a dry weight basis for water spinach (*Ipomoea aquatica*) selection 1 (A) and selection 2 (B) grown in 0.50 and 0.25 strength Hoagland no. 1 solutions.

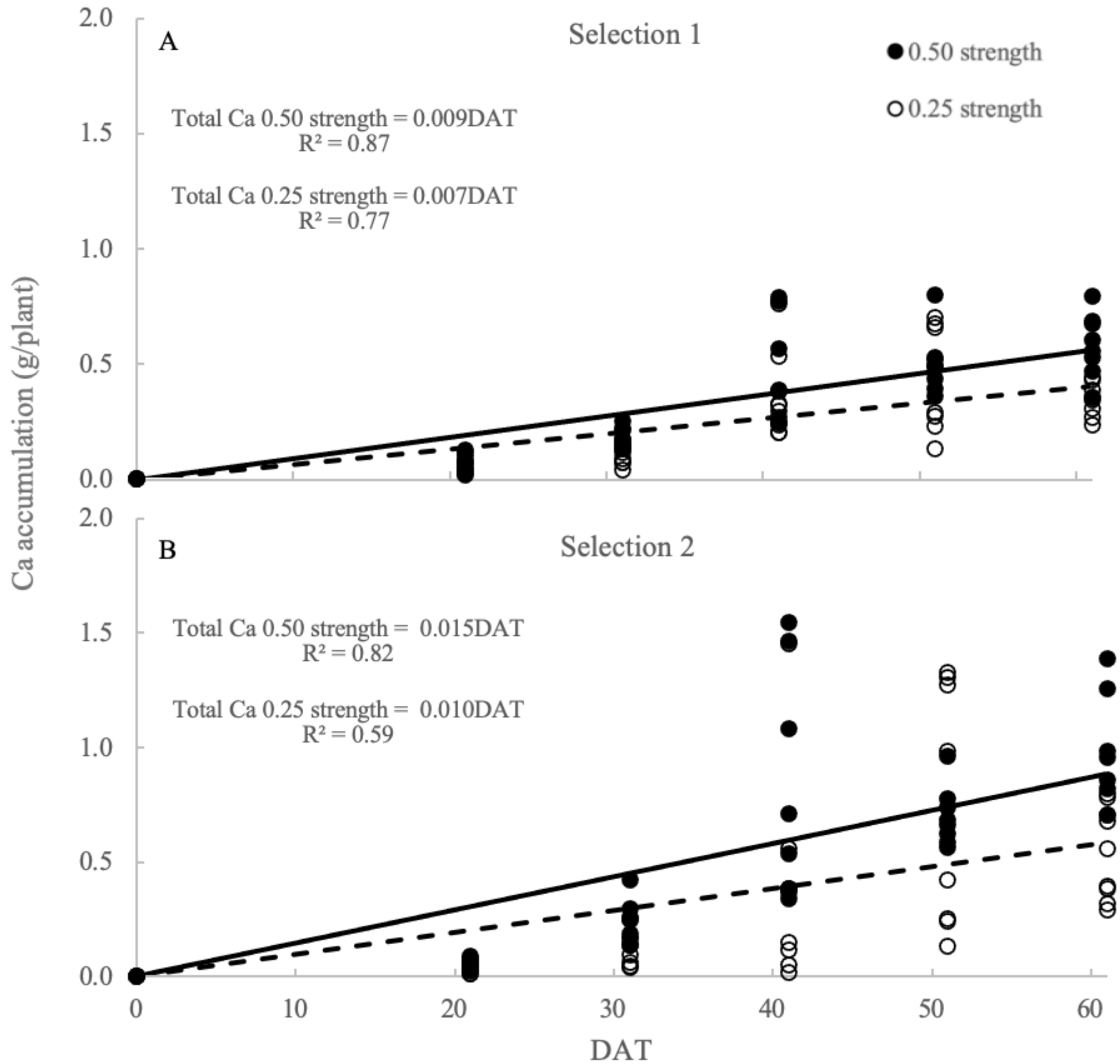
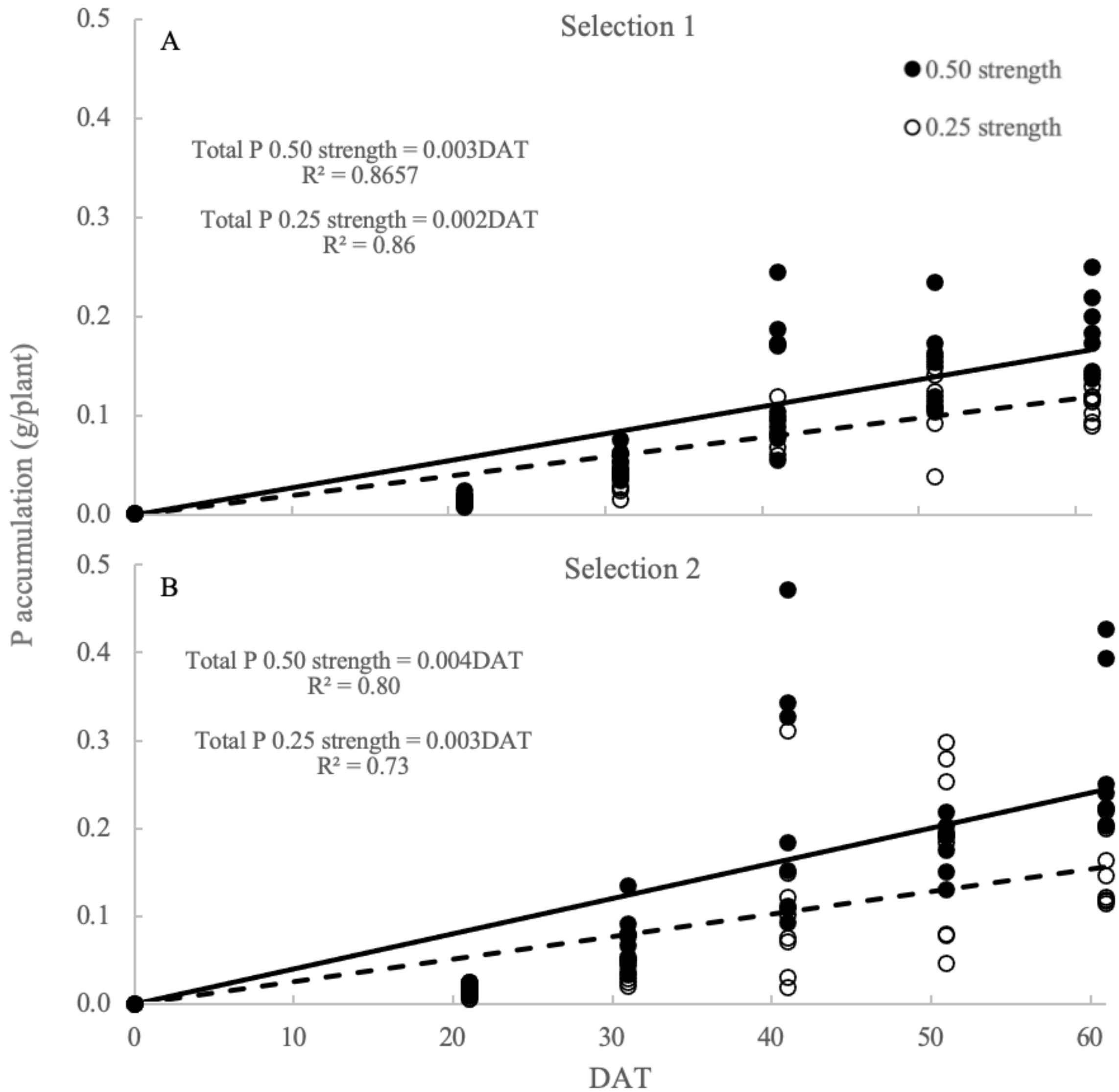


FIGURE 2.5 (A, B): Total calcium (Ca) uptake for root and shoot tissue on a dry weight basis for water spinach (*Ipomoea aquatica*) selection 1 (A) and selection 2 (B) grown in 0.50 and 0.25 strength Hoagland no. 1 solutions.

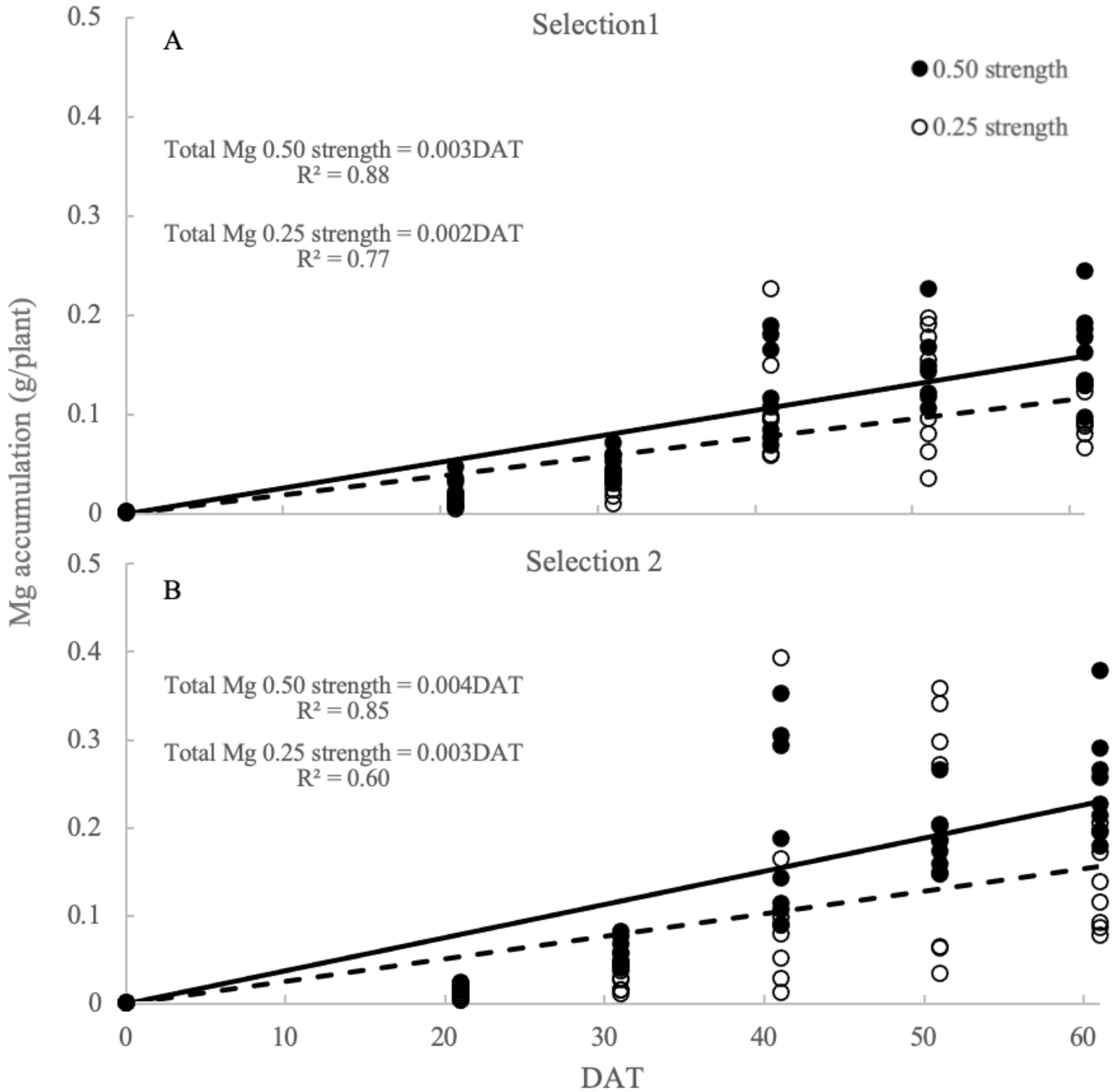
SUPPLEMENTAL TABLE S2.1: Nutrient concentration of shoots and roots in percent dry weight (DW) for phosphorous (P), magnesium (Mg) and sulfur (S) at different days after transplant (DAT) for hydroponically grown water spinach.

			DAT					
			0	21	31	41	51	61
		Nutrient Solution Strength	DW(%)					
			P					
Selection 1	Shoot	0.5	0.27 b[i]	0.38 ab	0.45 ab	0.41 ab	0.46 ab	0.50 a
		0.25	0.27 b	0.53 a	0.54 a	0.38 ab	0.40 ab	0.47 ab
	Root	0.5	0.27 a	0.40 a	0.34 a	0.29 a	0.31 a	0.40 a
		0.25	0.27 a	0.41 a	0.38 a	0.28 a	0.30 a	0.36 a
Selection 2	Shoot	0.5	0.26 c	0.53 b	0.53 b	0.45 b	0.43 bc	0.42 bc
		0.25	0.26 c	0.75 a	0.79 a	0.44 bc	0.45 bc	0.46 bc
	Root	0.5	0.26 a	0.43 a	0.36 a	0.34 a	0.27 a	0.26 a
		0.25	0.26 a	0.41 a	0.93 a	0.41 a	0.73 a	0.36 a
			Mg					
Selection 1	Shoot	0.5	0.92 a	0.34 b	0.38 b	0.39 b	0.43 b	0.43 b
		0.25	0.92 a	0.44 b	0.42 b	0.34 b	0.38 b	0.37 b
	Root	0.5	0.92 a	0.51 bc	0.62 b	0.52 bc	0.46 bc	0.47 bc
		0.25	0.92 a	0.55 bc	0.53 bc	0.38 c	0.39 bc	0.39 bc
Selection 2	Shoot	0.5	0.70 a	0.48 bcd	0.42 bcd	0.39 bcd	0.39 bcd	0.38 bcd
		0.25	0.70 a	0.53 b	0.48 bc	0.37 cd	0.32 d	0.32 cd
	Root	0.5	0.70 a	0.45 a	0.51 a	0.45 a	0.41 a	0.41 a
		0.25	0.70 a	0.41 a	0.81 a	0.39 a	0.77 a	0.39 a
			S					
Selection 1	Shoot	0.5	0.29 bcd	0.38 ab	0.28 bcd	0.24 d	0.29 bcd	0.30 bcd
		0.25	0.29 bcd	0.42 a	0.36 abc	0.26 d	0.26 d	0.28 cd
	Root	0.5	0.29 c	0.40 abc	0.36 abc	0.37 abc	0.30 bc	0.36 abc
		0.25	0.29 c	0.44 abc	0.50 a	0.47 ab	0.39 abc	0.42 abc
Selection 2	Shoot	0.5	0.33 c	0.42 ab	0.36 bc	0.31 c	0.31 c	0.33 c
		0.25	0.33 c	0.49 a	0.51 a	0.35 bc	0.32 c	0.34 bc
	Root	0.5	0.33 a	0.26 a	0.26 a	0.31 a	0.26 a	0.35 a
		0.25	0.33 a	0.25 a	0.59 a	0.33 a	0.70 a	0.38 a

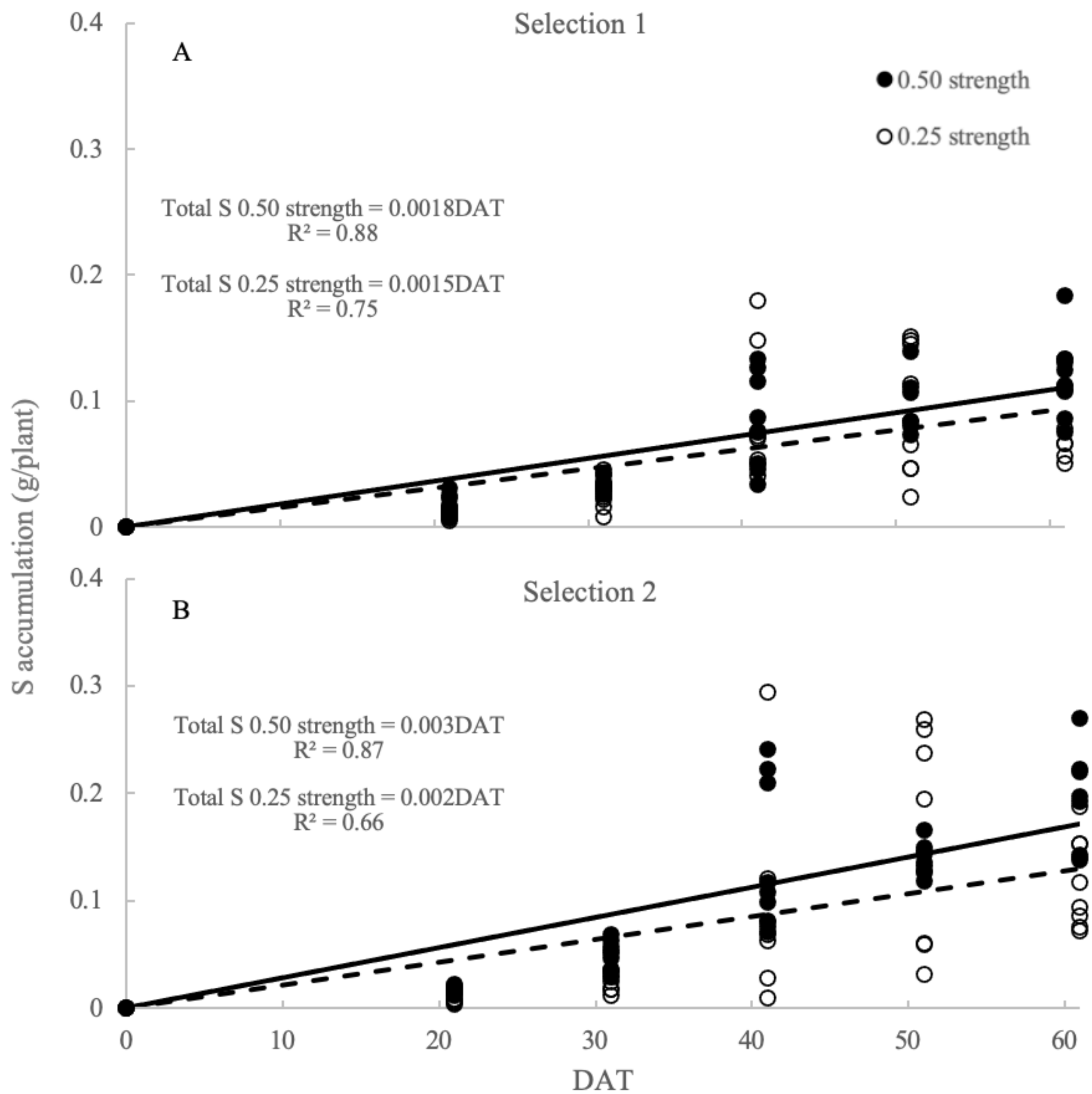
[i]Numbers followed by the same letter(s) indicate no significant difference according to the Tukey HSD test (P<0.05) when comparing shoot or root concentrations among the different nutrient solutions and sampling times for a single selection and nutrient



SUPPLEMENTAL FIGURE S2.1 (A, B): Total phosphorous (P) uptake for root and shoot tissue on a dry weight basis for water spinach (*Ipomoea aquatica*) selection 1 (A) and selection 2 (B) grown in 0.50 and 0.25 strength Hoagland no. 1 solutions.



SUPPLEMENTAL FIGURE S2.2 (A, B). Total magnesium (Mg) uptake for root and shoot tissue on a dry weight basis for water spinach (Ipomoea aquatica) selection 1 (A) and selection 2 (B) grown in 0.50 and 0.25 strength Hoagland no. 1 solutions.



SUPPLEMENTAL FIGURE S2.3 (A, B). Total sulfur (S) uptake for root and shoot tissue on a dry weight basis for water spinach (*Ipomoea aquatica*) selection 1 (A) and selection 2 (B) grown in 0.50 and 0.25 strength Hoagland no. 1 solutions

CHAPTER 3

BIOMASS AND NUTRIENT REMOVAL RATES FOR WATER SPINACH (*IPOMOEA AQUATICA*) GROWN IN A HIGH TUNNEL IN GEORGIA, USA[2]

[2] Bohensky, S. and Coolong, T. (2025) To be submitted for publication in HortTechnology.

Abstract

Water spinach (*Ipomoea aquatica*) is a leafy green vegetable that is routinely cultivated in Southeast Asia. Due to its aggressive growing habits and potential to invade waterways, it has been classified as a federal noxious weed within the United States. Ethnic communities have however shown significant interest in cultivation within certain U.S. states, leading to potential cultivation in Georgia, USA in the future. The purpose of this study is to develop recommendations for planting and harvest periods and fertilization recommendations for cultivation of water spinach in a high tunnel environment. Two selections of water spinach were grown in a certified organic high tunnel located in Watkinsville, Georgia USA. The two selections were planted on three dates (May, June, July) during summer 2023 in plots measuring 72ft^2 in a factorial randomized complete block design with four replicates. Once plants reached a harvestable size, the above-ground portions were cut near the base and this process was repeated during the growing season. The results of this study indicated that planting water spinach in May resulted in significantly greater yields compared to June and July plantings due to a greater number of harvests and biomass production at each harvest. The rate of fresh weight biomass production was greatest for the third harvest of the May planting, which was 1,764 lb/acre/day and 1,623 lb/acre/day for selections 1 and 2, respectively. Above ground nutrient concentrations and removal rates suggested that potassium (K) was found in the greatest concentration in shoot tissue. Total K removal for the May-planted water spinach was 851 lb/acre K and 778 lb/acre K for selections 1 and 2, respectively. These results suggest that planting in May can allow for multiple harvests over the growing season with multiple cuttings from a single planting. However, due to high biomass and K removal rates, plants may need supplemental applications of K when necessary.

Introduction

Water spinach (*Ipomoea aquatica*) is a commonly cultivated and consumed leafy vegetable throughout Southeast Asia and Southern China (Edie and Ho 1969; Rana and Brar 2018). Other commons names for water spinach include kang kong, swamp morning glory, and water convolvulus. It is an aquatic or semi-aquatic plant that grows well in tropical and sub-tropical climates particularly in regions where temperatures routinely exceed 25 ^{o}C. When grown in an aquatic environment, water spinach floats on the water's surface due to the hollow nature of the plant's stems, while their roots anchor the plant to riverbanks and shallow areas of waterways (Austin 2007; Edie and Ho 1969; Gangopadhyay et al. 2021). Water spinach thrives in high temperatures and provides an alternative to other leafy greens such as *Brassica* species, that may not be available during the hottest times of the year (Edie & Ho 1969). Typically, the young portions of the shoots are harvested for human consumption due to being the most tender part of the plant. These shoots can be incorporated into many cuisines such as stir fries or eaten by themselves cooked or raw. Older, less desirable, portions may be a significant source of nutrients and are often utilized as fodder for livestock (Ali and Kaviraj 2018; Kusumah and Pertiwi 2021; Maung et al. 2020; Sambo et al. 2023).

Although considered primarily an aquatic plant, water spinach may be cultivated on land as well. Production on land requires significant supplemental irrigation (Rana and Brar 2018). While irrigation is often provided through furrows or flooding, empirical evidence has shown that drip irrigation can also be utilized. Wetland cultivation may take place in natural waterways or in sunken fields where the plants may be flooded with water at a depth of 15-20cm (Rubtazky and Yamaguchi 1997; Snyder et al. 1981; Westphal 1993).

Water spinach is classified as a noxious weed and potentially invasive species in regions such as the United States (U.S.) and Mexico (Chilton 2017; Randall 2017; USDA 2020). When present in waterways, the hollow stems of water spinach may break off from the plants and float downstream in waterways or be relocated by humans and anchor into unwanted areas. Once established, water spinach is difficult to eradicate in climates where hard frosts and freezes are not present. The U.S., however, still legally allows water spinach to be cultivated in Florida, California, Texas, and Hawaii under strict guidelines (Chilton 2017; USDA 2020). Georgia, USA has recently allowed for water spinach to legally be imported and sold within the state, but at the time of this publication, production is still prohibited (Lee 2023; GDA 2022). Nonetheless due to demand from ethnic markets there are discussions underway that would allow water spinach to be cultivated within the state of Georgia, following strict guidelines to prevent unwanted spread of the plant (personal communication GDA). To avoid the spread of water spinach into natural waterways, it is likely that either greenhouse or dryland (soil based) cultivation will be recommended. In Texas, USA, most water spinach is grown using dryland cultivation in high tunnel or greenhouse structures, where it has been shown to be at low risk of invasiveness (Chilton 2017).

Water spinach thrives under tropical conditions with high air temperatures and frequent moisture (Rana and Brar 2018). Chilton (2017) reported that water spinach is grown throughout the summer in Texas, USA in greenhouses where temperatures may exceed 48 ^{o}C. Here, plants are harvested approximately every 10 d during the summer. Thus, water spinach may occupy a niche for high tunnel production during the summer months in the Southern U.S., when other vegetables may not grow well due to high temperatures.

Sarkar et al. 2014 compared three planting dates (May, June, July) and multiple plant spacings for water spinach grown for dryland production in the West Bengal region of India. They noted that the May planting with a 15 x 30 cm plant spacing produced the highest yield compared to plantings later in the summer months. While this study took place in West Bengal, India, summer weather conditions there are comparable to those found in Georgia, USA during the same period. That study suggested that planting date during the summer months impacted yield potential for water spinach. Therefore, the purpose of this study was to determine the yield and nutrient content of two varieties of water spinach planted at planting dates (May, June, July) to determine yield potential, harvest frequency and the optimal planting date for high tunnel water spinach production in Georgia, USA.

Materials and Methods

Experimental Setting

The study was conducted from May to Sep 2023 at the organic farming unit of The University of Georgia Durham Horticulture farm in Watkinsville, GA, USA (lat. 33^{o} 5'N, long. 83^{o} 3'W). The soil is a Cecil sandy loam series (0% to 2% slope). The study location has been certified organic since 2012 and all practices followed USDA National Organic Program Standards. The study was conducted in a high tunnel (Snow Arch; 30 ft x 90 ft, Atlas Greenhouse Systems Inc. Alapaha, GA, USA) covered with one layer of polyethylene plastic (6 mil, SunView 4; Poly-Ag Crop., San Diego, CA, USA). The tunnel had automated side curtains set to open when air temperatures in the tunnel reached 86 ^{o}F, measured approximately 6-ft above the soil line. Side curtains were closed again after a 10 ^{o}F differential (76 ^{o}F) had been reached.

Soils were sampled at a depth of 6 inches before planting (Mehlich 1 extract, University of Georgia Agriculture and Environmental Services Laboratory, Athens, GA). Soil pH was 6.7 with an average of 737 lb/acre phosphorous (P), 992 lb/acre potassium (K), and 7178 lb/acre calcium (Ca). Further, prior testing showed that soil organic matter was approximately 3.0%. Prior to the first planting date, the entire tunnel was tilled to a depth of approximately 6-8 inches using a tractor-mounted rotary tiller.

Seeds from two unnamed selections of water spinach were utilized for this experiment and hereafter are labeled as selection 1 (Gaea's Blessing, Austin, TX, USA) and selection 2 (Chiatai Seed, Bangkok, THA). Selection 1 had a hastate leaf type and is often utilized in production in China, while selection 2 had a lanceolate leaf type and is often found throughout Southeast Asia (Westphal, 1993) (Figure 3.1A, B). Seeds were sown in 200-cell styrofoam trays (Speedling, Ruskin, Fl, USA) using an organic seed soilless media (ProMix BX Organic; Premier Tech, Riviere-du-Loup, QC, CAN). Seedlings were grown for 21 d in a greenhouse with temperature set points of 28/20 ^{o}C (day/night).

On the day before planting, designated plots (12 ft x 6 ft) were fertilized with an organic pelletized fertilizer (10N-0.8P-6.6K; Nature Safe, Darling Ingredients Inc., Irving, TX, USA) at a rate of 100 lb/acre nitrogen (N). The fertilizer was applied and incorporated by hand. The experiment was arranged as a 3 x 2 factorial randomized complete block design with three planting dates and two selections of water spinach with four replicates of each combination of selection and planting date. Within each plot there were six rows containing six plants each. Seedlings were spaced 12 inches apart within a row and rows were 24-inches center to center. Seedlings were hand watered immediately after planting and drip irrigated subsequently thereafter. Drip tubing (12-inch emitter spacing, Toro Aquatrax, Toro, Bloomington, MN, USA) was placed equidistant (12 inches) from each planted row. Plants were irrigated daily to maintain adequate soil moisture throughout the experiment. Seedlings of selection 1 and 2 were planted 22 May, 29 Jun, and 29 Jul 2023 (Table 3.1).

Plants were grown until they reached a harvestable size (Table 3.1). At harvest, stems and foliage were cut near the base of the plant leaving several small leaves and shoots to re-sprout after harvest. Entire plots were harvested, and the total fresh weight (FW) and plant number recorded. Subsamples of 2 to 5 plants (aboveground portions only) were dried in a forced air oven set at 60 ^{o}C until a constant dry weight (DW) was achieved. Drying times varied due to variation in sample sizes and weights. Dried plant material was then coarsely ground for further nutrient analyses.

Nutrient Analysis

Dried shoots were weighed and finely ground. Dried plant material was analyzed by the University of Georgia Agriculture and Environmental Services Laboratory (Athens, GA, USA). In brief, coarsely ground plant material was ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) and passed through a 20-mesh screen. Samples were digested using EPA Method 3052 (USEPA, 1995). Plant tissues were analyzed for multiple elements following EPA Method 200.8 (Creed et al., 1994) by Inductively Coupled Plasma - Optical Emission Spectroscopy (Spectro Arcos FHS16; Spectro Amertek USA, Wilmington, MA, USA).

Environmental Data

Air temperature, relative humidity (RH), light levels, and volumetric water content (VWC) were measured in plots. The air temperature and RH of the high tunnel were monitored at canopy height (12 inches) (VP4; Meter Group Inc., Pullman WA, USA). Soil volumetric water content (VWC) was monitored with two probes placed approximately 5-6 inches below the soil surface equidistant between plants in a row and approximately 12 inches from drip irrigation tubing (10

HS, Meter Group Inc.). Sensors were connected to a data logger (EM 50G, Meter Group). Data from sensors were recorded every 15 min throughout the study.

Growing Degree Days

Growing degree days (GDD) were calculated from within the high tunnels using the following formula, $[(T_{max} + T_{min}) /2- T_{base}]$. Due to a lack of information for development of water spinach, GDD were calculated using a base temperature (T_{base}) of 15.5 ^{o}C and an upper limit for T_{max} of 32.2 ^{o}C based on GDD models developed for sweet potato (*Ipomea batatas*) (Villordon et al., 2009).

Statistical Analysis

Macronutrient concentrations of the dried shoot tissue were multiplied by the average plant dry weight for each plot and sampling time to determine nutrient accumulation. Statistical analysis was conducted using R statistical computing software. When statistical differences existed in the Analysis of variance (ANOVA), a Tukey's Honest Significant Difference (Tukey HSD) test ($P < 0.05$) was performed when necessary.

Results and Discussion

Average air temperature and RH in the high tunnel for the duration of the study were 25.4 ^{o}C and 72.3%, respectively. Average maximum and minimum air temperatures in the tunnel during the same period were 33.4 ^{o}C and 19.6 ^{o}C, respectively (Figure 3.2). Relative humidity ranged from 51% to 87% throughout the study. Soil moisture levels averaged 21.3% VWC for the study period (data not shown). Calculated GDD from planting until first harvest ranged from 390 to 537 (Table 3.1). For subsequent harvests, the GDD ranged from 271 to 339. This suggests that it may have taken more time for the plants to become established immediately after planting, but subsequently could be harvested after a shorter period of time. Harvest intervals once the crop was established for this study were similar to those previously reported in Florida, USA (Snyder et al., 1981). There is limited data for GDD and water spinach development; however, visual observations and harvest data suggest that as daily air temperatures decreased in late August and September (Figure 3.2), growth slowed. Observations by Chilton (2017) reported that water spinach was grown in Texas, USA in greenhouses with temperatures exceeding 48 ^{o}C, suggesting that the period 20 and 26 Aug. where air temperatures exceeded 32.2 ^{o}C, which was set as the upper limit for T_{max} (Figure 3.2) may not have been responsible for the reduced growth rate of the July-planted water spinach (Villordon et al. 2009).

Biomass

Biomass (FW) accumulation was significantly greater for all harvests for the May and June plantings compared to the July planting for selections 1 and 2 (Table 3.2). Selection 1 produced FW biomass totals of over 44,000 lb/acre for a single harvest interval. Interestingly, GDD accumulation did not correspond to FW yields for the water spinach. There were not significant differences between any of the harvests for yields for selection 1 planted in May or June, but GDD accumulation differed considerably (Table 3.1, 2). For both selections, the July planting had the lowest FW biomass accumulation, despite accumulating 537 GDD. This suggests that the yield of water spinach was likely affected by other environmental factors than temperature alone.

Because GDD and the number of days between harvests varied over the study, the FW and DW biomass accumulation rate were compared. Both FW and DW accumulation rates increased significantly with each successive harvest for both May and June planting periods in both water spinach selections (Table 3.3). For both selections, the third harvest of the May planting resulted in the greatest rate of FW biomass accumulation, though this was not significantly different from the second harvest from the May planting date. The total harvestable biomass of the May planting exceeded total biomass for June and July plantings. Planting seedlings in May allowed for three harvests over the summer growing period while planting in June resulted in two harvests and planting in July resulted in just one harvest. The rate of biomass accumulation for the July planting was roughly one-half of that for the first harvest of the May planting and approximately one-quarter for the first harvest of the June planting, despite having the largest accumulation of GDD of any harvest interval (Table 3.1). Due to the interactions of selection and planting date for biomass accumulation rate, the selections of water spinach were not compared directly to each other, but general trends were similar between the two for all planting and harvest dates. Similar findings were reported for water spinach grown in India, where planting in May resulted in the highest plant biomass harvested during the summer while planting in July resulted in the lowest plant biomass (Sarkar et al. 2014).

Nutrient Concentrations

Nitrogen concentrations decreased from the first to last harvest for the May planting in both selections (Table 3.4). The first harvest of the May planting had the highest concentration of N for selection 1, while the first harvest of the June planting resulted in the highest concentration of N for selection 2. This was significantly greater than the second harvest from both the May and June plantings. These results suggest decreased N concentrations as harvests continue throughout the season. This is not unexpected as there were no additional fertilizer applications during growth, and as plants mature and biomass increases, plant N concentrations often decrease (Jarrell & Beverly 1981).

Phosphorous concentrations for selection 1 were unaffected by planting date or harvest interval for selection 1 (Table 3.5). For selection 2, P concentrations tended to increase during the season, with the highest P concentrations being the second and third harvests of the June and May plantings, respectively. These results may suggest that more P is available for plant uptake as water spinach increases in biomass and maturity. Values for shoot P concentrations obtained in the present study were notably higher than those previously reported, rainging from 0.086% to 0.10% (Adedokun et al. 2019; Umar et al. 2007). Preplant soil levels averaged 737 lb/acre P, which was likely much greater than the levels plants were exposed to in prior studies.

Similar to N, K concentrations declined during harvest (Table 3.6). Values were similar between the two selections for the first harvest of the May planting, with both having a K concentration exceeding 11% (Table 3.6). Prior studies have reported that K was the most abundant macronutrient in water spinach shoot tissue (Amir et al. 2021; Umer et al. 2007). As with P, shoot K concentrations in the current study were greater than previously reported, but due to the high levels of preplant soil K (992 lb/acre K) that is not unexpected. Of all the nutrients measured in water spinach shoot tissue, K was the most abundant.

Nutrient Removal

Nitrogen accumulation and removal from the soil was similar in both selection 1 and selection 2 (Table 3.7). As expected, N removal increased as biomass increased for each harvest. The highest value of nitrogen removal for both selection 1 and 2 are from the third harvest of the May planting with an average removal of 108 lb/acre and 102 lb/acre, respectively. Because N concentrations in shoot tissue decreased significantly for these harvests (Table 3.4) the high rate of N removal was primarily a function of increased biomass production. Schulz et al. (2024) concluded similar results in high-yield sweet potatoes (*Ipomoea batatas*) where the removal rate for both the roots and foliage of the plants resulted in an average of 191 lb/acre N and the roots alone removed 98 lb/acre N. While sweet potatoes are harvested for their below-ground roots, water spinach displays similar growth habits as well as sharing the same *Ipomoea* genus. Nearly 284 and 256 lb/acre N were removed for selections 1 and 2, respectively, despite only 100 lb/acre N being applied in fertilizer. It is likely that the soils in these high tunnels, which have been in long-term organic production, had significant N mineralized from existing organic matter that contributed to the large amount of N removed from the soil at harvest.

Phosphorous removal in both selections had similar trends with the maximum average value being 29 lb/acre for selection 1 and 30 lb/acre for selection 2 (Table 3.8). Unlike N or K, P concentrations in the shoot tissue tended to increase from the first harvest to the second harvest and the increase in P removed resulted from both the increase in biomass production as well as the increase in P concentration during the study period. Similarly, a study conducted on high-yield sweet potato reported an average of 34 lb/acre removal of P from the roots and foliage of the plants (Schulz et al., 2024). Water spinach is popularly utilized in phytoremediation to remove nutrients such as P from waterways through absorption, though it may be more readily available to plants in an aquatic production system compared to the soil (Sa’at and Zaman 2017; Li et al. 2009).

Potassium accumulation in shoots was the largest of the nutrients analyzed in this study (Table 3.9). The removal trends for selections 1 and selection 2 were similar. Although not significantly different, the first harvest of the June planting for selection 1 had a K removal rate of 426 lb/acre K, which was 1.5 times larger than the second harvest with a removal of 261 lb/acre K. For May-planted water spinach, the total K removed for all three harvests were 851 lb/acre and 778 lb/acre K for selections 1 and 2, respectively. These results suggest that water spinach has the potential to accumulate and remove high amounts of K from the soil, which may be nutritionally important. Our results are considerably higher than those reported for sweet potato by Schulz et al. (2024) where 335 lb/acre K was shown to be removed from the soil from both roots and foliage of the plant while the root portion of the plant removed an additional 223 lb/acre K. Other studies of nutrient analyses on water spinach have indicated that K is most often the greatest macronutrient present within water spinach (Amir et al. 2021; Umar et al. 2007).

Conclusion

The results of this study suggest that water spinach can safely and effectively be grown within a high tunnel in soil. Differences in biomass as well as the number of harvests suggest that a single planting in late spring or early summer would be ideal for maximizing yield. This allowed for multiple harvests through the summer production season with significant production of biomass at each harvest. These results also suggest that supplemental applications of nutrients such as potassium may be required for optimal growth.

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TABLE 3.1: Planting, harvest dates and accumulated growing degree days (GDD) for water spinach grown in Watkinsville, GA, USA in 2023.

Planting Date	Harvest 1	Harvest 2	Harvest 3	Harvest 1	Harvest 2	Harvest 3
		Harvest Date			GDD	
22 May	7 Jul	1 Aug	26 Aug	390	271	301
29 Jun	15 Aug	16 Sep		521	339	
29 Jul	16 Sep			537		

[i](1.8 X oC) +32 = ºF
[ii]GDD = [$(T_{max} + T_{min} /2)$] – 15.5 ºC

TABLE 3.2: Fresh weight (FW) biomass (lb/acre) over time for each harvest interval for water spinach (Ipomoea aquatica) grown in a high tunnel in Watkinsville, GA, USA.

	Selection 1			Selection 2		
	May	June	July	May	June	July
			(lb/acre FW[i])			
Harvest						
1	41,019 a[ii]	44,286 a	13,977 b	21,666 bc	32,162 ab	9,202 c
2	37,026 a	38,115 a	--	29,149 ab	32,761 ab	--
3	44,105 a	--	--	40,565 a	--	--

[i]1 lb/acre =1.1209 kg·ha^{-1}.
[ii]Numbers followed by the same letter(s) indicate no significant difference according to the Tukey HSD test (P<0.05) when comparing biomass among the different harvest intervals for a single selection.

TABLE 3.3: Fresh weight (FW) biomass (lb/acre/day) and dry weight (DW) biomass (lb/acre/day) accumulation over time for each harvest interval for water spinach (Ipomea aquatica) grown in a high tunnel in Watkinsville, GA, USA.

		Selection 1			Selection 2	
		May	June	July	May	June	July
	(lb/acre/day FW[i])					
Harvest						
1	892 bc[ii]	942 b	285 c	471 cd	684 bcd	198 d
2	1481 ab	1191 ab	--	1166 ab	1024 bc	--
3	1764 a	--	--	1623 a	--	--
	(lb/acre/day DW)					
1	50 bc	100 abc	28 c	38 cd	66 bcd	18 d
2	129 ab	111 abc	--	116 ab	102 abc	--
3	159 a	--	--	135 a	--	--

[i] 1 lb/acre =1.1209 kg·ha^{-1}.

[ii] Numbers followed by the same letter(s) indicate no significant difference according to the Tukey HSD test ($P<0.05$) when comparing biomass among the different harvest intervals for a single selection.

TABLE 3.4: Nitrogen concentration over time for each harvest interval for water spinach (Ipomoea aquatica) grown in a high tunnel in Watkinsville, GA, USA.

	Selection 1			Selection 2		
	May	June	July	May	June	July
	N (%)					
Harvest						
1	3.62 a[i]	3.18 ab	3.21 ab	3.82 ab	4.22 a	3.42 abc
2	2.96 ab	2.84 ab	--	3.00 bc	2.72 cd	--
3	2.68 b	--	--	1.86 d	--	--

[i]Numbers followed by the same letter(s) indicate no significant difference according to the Tukey HSD test (P<0.05) when comparing nitrogen removal among the different harvest intervals for a single selection.

TABLE 3.5: Phosphorous concentration over time for each harvest interval for water spinach (Ipomoea aquatica) grown in a high tunnel in Watkinsville, GA, USA.

	Selection 1			Selection 2		
	May	June	July	May	June	July
			P (%)			
Harvest						
1	0.57 a[i]	0.64 a	0.59 a	0.53 c	0.56 bc	0.58 bc
2	0.79 a	0.78 a	--	0.59 bc	0.81 ab	--
3	0.79 a	--	--	0.88 a	--	--

[i]Numbers followed by the same letter(s) indicate no significant difference according to the Tukey HSD test ($P<0.05$) when comparing phosphorous removal among the different harvest intervals for a single selection.

TABLE 3.6: Potassium concentration over time for each harvest interval for water spinach (Ipomoea aquatica) grown in a high tunnel in Watkinsville, GA, USA.

	Selection 1			Selection 2		
	May	June	July	May	June	July
	K (%)					
Harvest						
1	11.35 a[i]	9.22 abc	6.58 c	11.53 a	8.22 bc	6.57 bc
2	9.26 ab	7.30 bc	--	9.43 ab	6.36 c	--
3	7.84 bc	--	--	9.12 abc	--	--

[i]Numbers followed by the same letter(s) indicate no significant difference according to the Tukey HSD test ($P<0.05$) when comparing potassium removal among the different harvest intervals for a single selection.

TABLE 3.7: Nitrogen (N) removal over time for each harvest interval for water spinach (Ipomoea aquatica) grown in a high tunnel in Watkinsville, GA, USA.

	Selection 1			Selection 2		
	May	June	July	May	June	July
			(lb/acre N[i])			
Harvest						
1	83 a[i]	151 a	47 a	66 ab	131 a	33 b
2	95 a	103 a	--	88 ab	91 ab	--
3	108 a	--	--	102 ab	--	--

[i]1 lb/acre =1.1209 kg·ha^{-1}.

[ii]Numbers followed by the same letter(s) indicate no significant difference according to the Tukey HSD test (P<0.05) when comparing nitrogen removal among the different harvest intervals for a single selection.

TABLE 3.8: Phosphorous removal over time for each harvest interval for water spinach (Ipomoea aquatica) grown in a high tunnel in Watkinsville, GA, USA.

	Selection 1			Selection 2		
	May	June	July	May	June	July
			(lb/acre P[i])			
Harvest						
1	13ab[ii]	28 a	8 b	9 cd	17 bc	5 d
2	25 ab	27 a	--	16 bc	27 ab	--
3	29 a	--	--	30 a	--	--

[i]1 lb/acre =1.1209 $kg\cdot ha^{-1}$.

[ii]Numbers followed by the same letter(s) indicate no significant difference according to the Tukey HSD test ($P<0.05$) when comparing phosphorous removal among the different harvest intervals for a single selection.

TABLE 3.9: Potassium removal over time for each harvest interval for water spinach (Ipomoea aquatica) grown in a high tunnel in Watkinsville, GA, USA.

	Selection 1			Selection 2		
	May	June	July	May	June	July
	(lb/acre K[i])					
Harvest						
1	255 ab[ii]	426 a	90 b	204 ab	260 a	57 b
2	297 ab	261 ab	--	265 a	200 ab	--
3	299 ab	--	--	309 a	--	--

[i]1 lb/acre =1.1209 kg·ha^{-1}.

[ii]Numbers followed by the same letter(s) indicate no significant difference according to the Tukey HSD test ($P<0.05$) when comparing potassium removal among the different harvest intervals for a single selection.

FIGURE 3.1 (A, B): Two selections of water spinach (Ipomoea aquatica) with varying leaf structure. A) Hastate leaf type that is more common in China, Selection 1. B) Lanceolate leaf type that is more common in Southeast Asia, Selection 2.

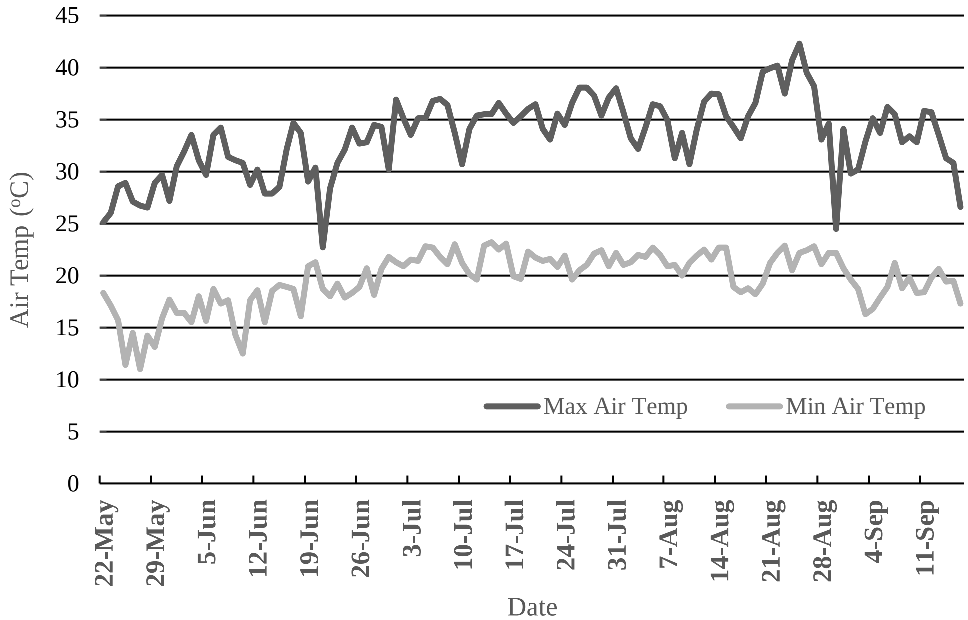

FIGURE 3.2: Minimum and Maximum air temperatures within the high tunnel over the growing period.

CHAPTER 4

CONCLUSIONS

Water spinach is a popular leafy green vegetable throughout Southeast Asia where it is a staple component in many dishes. Due to its popularity, some states within the U.S. have been legally permitted to cultivate water spinach, although information on production practices are minimal. The Asian community that is primarily located in the metropolitan region of Atlanta, Georgia, USA has been petitioning for the cultivation of water spinach within the state for many years. Currently, water spinach can legally be imported and sold while cultivation within the state is expected to be permitted in the coming years. The main concern for cultivating water spinach in the U.S. is that it is classified as a federal noxious weed by the USDA due to its invasive growing habit. Water spinach has the ability to take over waterways in a matter of months with its fast-growing shoots and nodes that can effectively root when fragments are broken off. Because of this, U.S. growers must have a legal permit and follow strict guidelines when cultivating water spinach such as growing indoors where the plants cannot escape. However, due to these cultivation limitations, there is minimal information for growers within the U.S. on how to properly cultivate water spinach. The objective of this thesis was to develop cultural practices for the production of water spinach in Georgia, USA where it could be grown safely and effectively. This research was undertaken to determine the nutrient requirements of water spinach as well as planting dates and harvest expectations.

Macronutrient uptake and accumulation for water spinach was determined in chapter 1 through a deep-water hydroponic culture system so that we could begin to develop clear guidelines for fertilizer recommendations in a hydroponic production system. We found that in regard to nutrient solution rates, the two selections of water spinach used in this study responded differently. Selection 2, which has a lanceolate-leaf type, displayed more of a positive response to the increased nutrient concentration strength in the 0.50 strength Hoagland's solution. With that being said, both selections had a positive response to the 0.50 strength Hoagland's solution compared to the 0.25 strength. This suggests that the greater strength solution should be considered as a baseline in a hydroponic production system. Over the 61 day harvest period, 2 plants were removed from each basin every 10 days. Commercial growers will not be removing full plants from the hydroponic systems; therefore, it would be recommended to increase concentrations of some nutrients over the growing period as plants grow larger to make up for this. It is also important to note that a full 61 days of growth typically should not be required in order to harvest water spinach. Upon observations, harvestable size was met at approximately day 41 where all shoots could be removed, and roots would remain in the hydroponic system as a cut and come again crop.

Biomass and nutrient removal rates were determined in chapter 2 through a trial evaluating three separate planting dates within a high tunnel. Through this study, we found that planting water spinach seedlings in the month of May led to a greater number of harvests and a significant production of biomass along with each harvest. By planting in May, three harvests with a total of 4,137 lb/acre/day and 3,260 lb/acre/day for selections 1 and 2, respectively were possible. The above-ground portions of water spinach harvested during this study suggest that the greatest nutrient concentration found in the plant tissue is potassium (K). From the three harvests of the May planting, 851 lb/acre K and 778 lb/acre K for selections 1 and 2 were removed from the soil over this one growing season. These results suggest that growers may need to provide supplemental applications of K during the growing season. The justification behind planting in May, June, and July was due to the fact that water spinach requires relatively high temperatures for optimal growth. For the May planting date, we postponed planting to allow for a rise in temperatures, which is why these seedlings were planted relatively late in the month (22nd). The last frost date in the Georgia, USA is typically at the end March, which leads us to believe that water spinach could be safely planted earlier in the month of May or late April to further extend the growing season if temperatures are high enough. During this growing season, we believe that the ideal size chosen for our harvests may have been too large, suggesting that planting in May could result in an even greater number of harvests.

In both studies, water spinach plants exhibited aggressive growth. Selection 1, which has a vining growth habit, tended to trail much further from their stationary roots and wrap around objects. Selection 2, while having more of an upright growing tendency, still managed to trail when planted in the high tunnel. When either selection’s shoots trailed across the soil of the high tunnel, nodes were fairly quick to root into the soil and create new plants when attempts of removal were unsuccessful. For growers, an option would be to apply weed barrier fabric to the entire surface of the high tunnel with holes present for seedlings if a uniform plot is wanted. Common pests found on both selections during this study were aphids, armyworms, and cutworms, so routine pesticide applications may be necessary. Furthermore, nearing the end of both studies in the month of Sep 2023, *Cercospora* leaf spot was detected on the leaves of plants, leading to a decrease in quality.

In conclusion, our results suggest that water spinach can be safely and effectively cultivated in the state of Georgia, USA when grown in a controlled environment to prevent unwanted spread. It is important to implement production practices that limit the potential spread of this plant and only cultivate if legally permitted by the USDA.